ANTIARTHIRITIS AND INVIVO ANTI-OXIDANT EFFECT OF *PLUMBAGO ZEYLANICA* LINN IN FREUND'S COMPLETE ADJUVANT INDUCED ARTHIRITIS

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CERTIFICATE

This is to certify that the dissertation entitled **"ANTI ARTHIRITIS AND INVIVO ANTIOXIDANT EFFECT OF *PLUMBAGO ZEYLANICA* LINN IN FREUD'S COMPLETE ADJUVANT INDUCED ARTHIRITIS",** submitted by **Mr.C.YOGESH PRABHU** in partial fulfillment for the degree of **"Master of Pharmacy in Pharmacology"** under The Tamilnadu Dr. M.G.R Medical University Chennai, at **K.M.College of pharmacy**, Madurai–107, is a bonafide work carried out by him under my guidance and supervision during the academic year of **2014 – 2015**. This dissertation partially or fully has not been submitted for any other degree or diploma of this university.

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CHAPTER - I

Introduction

INTRODUCTION

Inflammation

Inflammation is a normal, essential, and protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complex response involving the whole organism.

Many substances called mediators are formed or realized either concurrently or in successive time sequences at the site of injury. Various cell sources are responsible to an etiological factor. Various cells containing potent mediators and in some instances, inhibitors of the inflammatory response. These cell sources may include neutrophils, basophils, mast cells, platelets, macrophages and lymphocytes. The mediators of inflammation implicated in the inflammatory process and elaborated by the foregoing cells include histamine, serotonin, plasmakinins, lymphokines and prostaglandins **(1) Davis and Granner, 1996)** the process of inflammation can be summarized as follows.

- Initial injury to tissues causing release of mediators, histamine, serotonin, prostaglandins.
- An acute transient phase characterized by local vasodilatation and increased capillary permeability.
- A delayed sub acute phase, most prominently characterized by infiltration of leukocytes and phagocytic cells.
- A chronic proliferative phase, in which tissue degeneration and fibrosis occurs.

Conditions of inflammation

Many of the world's major diseases like infection, cancer, autoimmunity and allergy, critically involve the inflammation. Continued progress in understanding basic mechanisms of inflammation is essential for developing new abilities to treat and prevent diseases that affect millions worldwide.

Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease, the pathology of which is primarily and symmetrically localized in diarthrodial joints. The pathogenesis of RA is characterized by an inflamed synovium (lining the joint cavity), degradation of articular cartilage and erosion of sub-chondral bone. The systemic ramifications of the disease, with their attendant morbidity and mortality, include cardiopathy, nephropathy, vasculopathy and pulmonary and cutaneous disorders **(2)Firestein., 2001**). The prevalence of RA in India is quite similar to that reported from the developed countries. Projected to the whole population, this would give a total of about seven million patients in India. It is higher than that reported from China, Indonesia, Philippines and rural Africa. **(3)Malaviya *et al.*, 1993**). Although the cause of RA is unknown, the presentation of anarthritogenic self antigen to a genetically susceptible individual is believed to trigger the activation of auto-immunological pathways that lead to RA. Intense investigation of the cause of RA has uncovered many of the integral biochemical, cellular and molecular pathological components and pathways of this disease, leading to the discovery, development and marketing of new and novel therapeutics that target several seminal components of RA **(2)Firestein, 2001**). Thus, arthritis is an autoimmune disease in which inflammation is a predominant feature. Inflammation is the reactive state of hyperemia and exudation from blood vessels with consequent redness, heat, swelling and pain which a tissue manifests in response to physical or chemical injury or bacterial invasion. The present investigation was designed to unravel the analgesic, anti-inflammatory & antiarthritic activity hydroalcoholic extract of *Plumbago zeylanica* in various animal models.

Inflammation:

Inflammation is nature's double-edged sword. Inflammation characterized by pain and swelling, is triggered as a healing response in the body when it is injured or attacked by negative bacteria and viruses. Once the body recovers, the inflammation goes away. Obviously, dark side of inflammation, inflammation that doesn't heal, that doesn't go away, is one of the most prevalent health problems today. Dangers of Conventional Treatment for Inflammation

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen.

In pathologic conditions such as non-healing pressure ulcers, this efficient and orderly process is lost and the ulcers are locked into a state of chronic inflammation characterized by abundant neutrophil infiltration with associated reactive oxygen species and destructive enzymes. Healing proceeds only after the inflammation is controlled. On the opposite end of the spectrum, fibrosis is characterized by excessive matrix deposition and reduced remodeling. Often fibrotic lesions are associated with increased densities of mast cells. By understanding the functional relationships of these biological processes of normal compared to abnormal wound healing, hopefully new strategies can be designed to treat the pathological conditions.

Comparison between acute and chronic inflammation:

	Acute	**Chronic**
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions
Major cells involved	Neutrophils, mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary mediators	Vasoactive amines, eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Healing, abscess formation, chronic inflammation	Tissue destruction, fibrosis

The characteristics of inflammation can be divided into acute, chronic, irritability and immunity related inflammation. Any factor that induces tissue damage could be described as the pathogenesis of an inflammation. There are two kinds of induced inflammation factors: the inflammation stimulation factors, which mainly include physical (e.g., bruises, burns, frostbite, radial damage, etc.) and chemical factors (acid, alkali, allergens, mineral oil, etc.), and biochemical factors (microorganisms, parasites, endotoxins, transplant heterogeneity and animal toxins). Other inflammatory media include histamine, bradykinin, prostaglandin, platelet activation factor, neutrophils hydrolase, inflammation prestimulation factors (TNF, IL-1, IL-6, cell chemotaxis factor, etc.), adherence cell (select element, conformity element, adherence cell between cells, blood vessel cell adherence cell, etc.), cruor system stimulation factor, acute reaction protein (C reaction protein, LPS combined protein, serum starched protein A, etc.) **(4)Akarasereenont *et al*., 1995).** So inflammation is nature's double-edged sword.

Causes of Inflammation

Various agents may kill or damage cells as: Physical (heat or cold, Trauma, radiation), chemical (simple chemical poisons e.g.: acid, organic poisons e.g.: paraquat), Infection (bacteria, virus), Immunological (antigen – antibody, cell mediated)

Pathology of Inflammation

The classical signs of inflammation are: Redness (Rubor), Swelling (Tumor), Heat (color), Pain (Dolor), Loss of Function.

Distinct phases of inflammatory responses

- First phase is caused by an increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space.
- The second phase involves the infiltration of leukocytes from the blood into the tissue.
- Granuloma formation and tissue repair.

Distinct phases of inflammatory response

Vasodilatation and increased permeability of blood vessels

Within minutes after an injury, dilatation of arterioles and increased permeability of capillaries produce heat, redness and swelling in the affected area. The large amount of warm blood flowing through the area produces both heat and redness. Edema results from increased permeability of blood vessels, which permits more fluid to move from blood into tissue spaces, pain whether immediate or delayed is cardinal symptom of inflammation; it can result from injury of nerve fibres or from irritation by toxic chemicals from micro-organisms. Kinins affect some nerve endings causing much of the pain associated with inflammation.

Exudation of leukocytes

Changes in the formed elements of blood

In the early stage of inflammation, the rate of flow of blood is increased due to vasodilatation. But subsequently, there is slowing or stasis of blood stream. With stasis, changes in the normal axial flow of blood in the microcirculation take place. Due to slowing and stasis, the central stream of cells widens and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as margination. As a result of this redistribution, the neutrophils of the central column come close to the vessel wall, this is known as pavementing.

Adhesion

In this, peripherally marginated and pavemented neutrophils stick briefly to the endothelial cells lining the vessel wall or roll over it.

Emigration

After sticking of neutrophils to endothelium, they begin to squeeze through the wall of the blood vessel to reach the damaged area. This process is known as emigration. Simultaneous to emigration of leukocytes, escape of red cells through gaps between the endothelial cells, diapedesis takes place.

Chemotaxis

The chemotactic factor mediated transmigration of leukocytes after crossing several barriers to reach the interstitial tissues is called chemotaxis.

Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells. There are 2 main types of phagocytosis cells.

- Polymorphonuclear neutrophils
- Macrophages

Phagocytosis involves the following 4 steps:

1. Attachment stage
2. Engulfment stage
3. Secretion stage
4. Killing stage

Mediators of inflammation

Plasma derived mediators:

Name	Produced by	Description
Bradykinin	*Kinin system*	A vasoactive protein which is able to induce vasodilation, increase vascular permeability, cause smooth muscle contraction, and induce pain.
C3	*Complement system*	Cleaves to produce *C3a* and *C3b*. C3a stimulates histamine release by mast cells, thereby producing vasodilation. C3b is able to bind to bacterial cell walls and act as an opsonin, which marks the invader as a target for phagocytosis.

C5a	*Complement system*	Stimulates histamine release by mast cells, thereby producing vasodilation. It is also able to act as a chemoattractant to direct cells via chemotaxis to the site of inflammation.
Factor - XII (*Hageman Factor*)	*Liver*	A protein which circulates inactively, until activated by collagen, platelets, or exposed basement membranes via conformational change. When activated, it in turn is able to activate three plasma systems involved in inflammation: the kinin system, fibrinolysis system, and coagulation system.
Membrane attack complex	*Complement system*	A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The combination and activation of this range of complement proteins forms the *membrane attack complex*, which is able to insert into bacterial cell walls and causes cell lysis with ensuing death.
Plasmin	*Fibrinolysis system*	Able to break down fibrin clots, cleave complement protein C3, and activate Factor XII.
Thrombin	*Coagulation system*	Cleaves the soluble plasma protein fibrinogen to produce insoluble fibrin, which aggregates to form a blood clot. Thrombin can also bind to cells via the PAR1 receptor to trigger several other inflammatory responses, such as production of chemokines and nitric oxide.

Cell derived mediators:

Name	Type	Source	Description
Lysosome granules	*Enzymes*	Granulocytes	These cells contain a large variety of enzymes which perform a number of functions. Granules can be classified as either *specific* or *azurophilic* depending upon the contents, and are able to break down a number of substances, some of which may be plasma-derived proteins which allow these enzymes to act as inflammatory mediators.
Histamine	*Vasoactive amine*	Mast cells, basophils, platelets	Stored in preformed granules, histamine is released in response to a number of stimuli. It causes arteriole dilation and increased venous permeability.
IFN-γ	*Cytokine*	T-cells, NK cells	Antiviral, immunoregulatory, and anti-tumour properties. This interferon was originally called macrophage-activating factor, and is especially important in the maintenance of chronic inflammation.
IL-8	*Chemokine*	Primarily macrophages	Activation and chemoattraction of neutrophils, with a weak effect on monocytes and eosinophils.

Leukotriene B4	*Eicosanoid*	Leukocytes	Able to mediate leukocyte adhesion and activation, allowing them to bind to the endothelium and migrate across it. In neutrophils, it is also a potent chemoattractant, and is able to induce the formation of reactive oxygen species and the release of lysosome enzymes by these cells.
Nitric oxide	*Soluble gas*	Macrophages, endothelial cells, some neurons	Potent vasodilator, relaxes smooth muscle, reduces platelet aggregation, aids in leukocyte recruitment, direct antimicrobial activity in high concentrations.
Prostaglandins	*Eicosanoid*	Mast cells	A group of lipids which can cause vasodilation, fever, and pain.
TNF-α and IL-1	*Cytokines*	Primarily macrophages	Both affect a wide variety of cells to induce many similar inflammatory reactions: fever, production of cytokines, endothelial gene regulation, chemotaxis, leukocyte adherence, activation of fibroblasts. Responsible for the systemic effects of inflammation, such as loss of appetite and increased heart rate.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory disease of the joints but may affect other organ systems. **(5)Douglas *et al* 2001).** RA is characterized by Synovial proliferation as well as inflammatory and immunological processes. These mechanisms lead to largely irreversible degradative and erosive changes in the articular cartilage and juxtaarticular bone, and to a systemic disturbance of bone remodeling, finally resulting in systemic osteopenia. Initially, RA most commonly affects the small joints of the hand and wrist in a symmetrical fashion, and it has a predilection for the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints in the first instance. The distal interphalangeal (DIP) joints are spared in the vast majority of cases. It may occasionally present, however, as a monoarthritis of a large joint, such as the knee or shoulder. It is an autoimmune disease, which is defined clinically by the pattern of joint involvement and synovial swelling which is often described as 'boggy' due to the inflammatory nature of the synovial pathology. It may also be associated with the presence of circulating rheumatoid factor in the serum in 85% of patients, this is most commonly an IgM antibody directed against an IgG molecule. The test for rheumatoid factor is of some diagnostic use. However as it is not always present, especially in early disease, RA should not be excluded on the basis of a negative test. The rheumatoid hand is therefore critical in the assessment of patients suspected of early or recent onset RA. If we are to prevent joint damage, limit deformity and thereby preserve function it is imperative to identify the 'rheumatoid hand' before it develops the well described characteristic anatomical changes of longstanding disease. These changes include boutonniere or swan-neck deformities, ulnar deviation of the fingers and subluxation of the MCP and wrist joints.

In recent years, algorithms have been developed to identify individuals at high risk of RA, based on clinical features such as gender, family history and laboratory investigations such as C reactive protein, rheumatoid factor and even genetic typing using the shared epitope. It has been demonstrated that the third hypervariable region of the HLA DR4molecule is associated with RA, although it remains unclear whether it is a marker for susceptibility or severity. Recent studies show that clinical examination of the rheumatoid hand is relatively poor at detecting low grade or early

synovitis and therefore more sensitive diagnostic methods are needed. Diagnostic approaches in early disease have been under intense focus in the last10 years as rapid technological development of imaging techniques such as high resolution ultrasound (HRUS) and magnetic resonance imaging (MRI) offer specific advantages over conventional techniques. Therapeutic strategies have also changed rapidly over the past 20 years, and most recently have been transformed with the introduction of combination therapeutic approaches and the development of biologic agents. **(5) Douglas *et al* 2001)**.

The therapeutic approach affords symptomatic relief but the process of degeneration of the cartilage is not arrested by the drugs like NSAIDS. There is paucity of disease modifying drugs. However in the traditional system of medicine large no of plants have been reported to afford relief in the symptoms of rheumatoid arthritis. The polyphenols from plants are now investigated due to their antioxidant properties for Antiarthritic activity.

Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact. Epidemiology of the arthritis in female:male is 3:1 and the prevalence is 1% of the world population**. (6)Narendhirakannan *et al* 2007).** In human as well as in animal models, RA is characterized by a series of pathological processes of the joints, such as leukocyte infiltration, a chronic inflammation, pannus formation, and extensive destruction of the articular cartilage and bone. Although the exact cause of RA has not been elucidated in detail, pro- and anti-inflammatory cytokines seem to play an important role in the etiology of the disease. In particular, it was reported that the inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)- 1β, and IL-6, play key roles in the inflammation and joint damages during the development of RA **(7)Yeom *et al* 2006)**

ETIOLOGY

The cause of RA is thought to be a mixed result of genetic and environmental factors. Genetics determines about 50% to 60% of the susceptibility severity, and phenotypes for RA Monozygotic twins have concordance rates of 15%, while dizygotic rates fall around 4%. The association with HLA locus types has been well studied. Recently, a shared epitope within the third hypervariable region of HLADRB1 *0401 and *0404 alleles was reported to indicate more susceptibility and more severe disease. Susceptibility and severity are greater in men younger than 30 years with this genotype, even though young men as a whole have a reduced risk of RA. Homozygote's for these alleles are at an increased risk of severe disease, especially in younger men. However, showed an almost equivalent RA risk in men and women homozygous for the shared epitope . The risk for RA increases with more shared epitope involvement. 80% have at least 1 shared epitope and 42% have 2. The strongest association is seen with the *0401/0404 genotype. The combination of male gender and having 1 or 2 shared epitopes correlates directly with worse outcome. Shared epitope disease is associated with a younger age of onset, more joint tenderness, swelling, deformity, and RF positivity.HLA-DRw3 appears to be associated with greater RF positivity, rheumatoid nodules, and overall disease severity. HLA-DRw4 is linked to a family history of disease (77% versus 34% in control subjects). HLA-DRw3 and -DRw2 genotypes have also been shown to increase the chance of toxic reactions to RA therapy. HLA-DRw2 allele, however, appears to be a protective factor; it is seen less in RA patients and its presence is associated with fewer rheumatoid nodules and lower RF titers in patients. Non-HLA based genetic associations, especially on chromosomes 1p, 1q, and 18q, is also noted and are based on a combined analysis of 512 multicase families. Nongenetic components include aging, acquired genomic variability, possible bacterial or viral triggers, and other environmental factors. Smoking is the only lifestyle factor determined to be related to RA development. Aging is a risk factor for RA, as age-adjusted incidence per 100,000 persons is greatest among persons between 60 and 65 years of age. The illness may be associated with Epstein- Barr virus and Escherichia coli infections, since they have cross-reactive molecular links to the hypervariable domains of HLA DRDw4 and HLA-DRB1 seen in RA patients, **(8)Sayah *et al* 2005)**

PATHOGENESIS

RA appears to develop from a deregulated immune response that leads to progressive synovial inflammation and joint destruction. Heretofore, precipitating factors for the development of RA have not been elucidated fully, though T-cell involvement has been strongly implicated. CD4 T cells characteristically are infiltrating the synovium, a finding that suggests the process is mediated by T cells. Cytokines, primarily interleukin-1andtumornecrosis factor-a, and, recently reported, activation-induced, T-cell derived, chemokine-related cytokine/lymphotactin and macrophage migration inhibitory factor have been found in high levels in RA patients. These endogenous compounds stimulate synovial tissue effector functions, including proliferation, metalloproteinase expression, adhesion-molecular expression, secretion of other cytokines, and prostaglandin production—all of which may have a role in RA pathogenesis. Matrix metalloproteinase cause cartilage and bone degradation by means of extracellular matrix remodeling and degradation. Aberrant adhesion molecule expression leads to uncontrolled binding of T cell synovial type B cells, resulting in excess release of matrix metalloproteinase's. Other possible actors include neoangiogenesis, cyclo-oxygenases, nitric oxide synthase, and neutral proteases.4 RF is a group of auto antibodies (IgM, IgG, and IgA) that recognizes the Fc portion of IgG. It can be found in healthy persons during an immune response and in patients with various autoimmune diseases, as well as in those with chronic bacterial infections. Normally RF aids in the clearing of immune complexes, the processing of antigens by B cells, and the development of an early antibody repertoire. Increases in RF production commonly are seen prior to the clinical onset of RA. Various theories exist as to the origin of RF. They include triggering by IgG-antigenebased immune complexes, foreign antigen or autoantigen cross reactivity, anti-idiotype mimicry of exogenous Fc binding proteins (such as Staphylococcus aureus protein A), polyclonal B-cell activation by infections, and Fc glycosylation changes. There also appears to be a class switch from IgM to IgG, making it more pathologic. The antigen that triggers T-helper cells to aid in the switch is yet unknown. Although high levels of both IgM and IgG correlate with RA activity, increases in IgM and IgA together have been shown to have a strong specificity and predictive value for RA. These antibodies are heterogeneous and appear to change forms throughout the disease course, progressively increasing affinity for the Fc region. Pathogenic RF differs from that

seen in healthy persons in isotope, specificity, mutation frequency, and the ability to activate complement. The complete role of RF in RA pathogenesis is unclear. One part of the autoimmune response to RA may be due to the development of RF complexes. These complexes fix complement, recruit macrophages, neutrophils, and lymphocytes, and release cytokines upon ligation of Fc-gamma receptors on macrophages that together lead to inflammation. There is also a correlation between RF at baseline and future bone erosions. Specifically, IgA RF may lead to excessive intra-articular tissue growth factor-b secretion, creating the bone erosions seen in RA. As a marker of disease, RF correlates well with C-reactive protein but not erythrocyte sedimentation rate (ESR). Titers also correlate with arthritis severity and joint destruction, as well as vasculitis. **(8)Sayah *et al* 2005)**

TREATMENT

DESIRED OUTCOME;

The primary objective is to improve or maintain functional status there by improving quality of life. Treatment of rheumatoid arthritis is a multifaceted approach that includes pharmacologic and nonpharmacologic therapies. Recent emphasis has been placed on aggressive treatment early in the disease course. The ultimate goal is to achieve complete disease remission, although this goal is seldom achieved. Additional goals of treatment include controlling disease activity and joint pain, maintaining the ability to function in daily activities or work, improving the quality of life, and slowing destructive joint changes. **(9)Dipiro *et al* 2005)**

NONPHARMACOLOGIC THERAPY;

Rest, occupational therapy, physical therapy, use of assistive devices, weight reduction, and surgery are the most useful types of nonpharmacologic therapy used in patients with rheumatoid arthritis. Rest is an essential component of a nonpharmacologic treatment plan. It relieves stress on inflamed joints and prevents further joint destruction. Rest also aids in alleviation of pain. Too much rest and immobility, however, may lead to decreased range of motion, and ultimately muscle atrophy and contractures. Occupational and physical therapy can provide the patient with skills and exercises necessary to increase or maintain mobility. These disciplines

also may provide patients with supportive and adaptive devices such as canes, walkers, and splints. Other nonpharmacologic therapeutic options include weight loss and surgery. Weight reduction helps to alleviate stress on inflamed joints. This should be instituted and monitored with close supervision of a health care professional. Tenosynovectomy, tendon repair, and joint replacements are surgical options for patients with rheumatoid arthritis. Such management usually is reserved for patients with severe disease**. (9)Dipiro *et al* 2005)**

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Because of their analgesic and anti-inflammatory properties, nonsteroidal anti-inflammatory drugs (NSAlDs) are basic therapy for patients with RA. High doses and scheduled dosing may be required to achieve sufficient anti-inflammatory activity with NSAlDs in these patients. NSAlDs provide analgesia and suppress inflammation by inhibiting the enzyme cyclooxygenase (COX), resulting in decreased prostaglandin synthesis. The suppression of prostaglandin synthesis can also produce gastric and renal toxicity, as well as impair normal platelet function.COX exists in 2 isoenzymatic forms, COX-1 and COX-2. COX-1 is constitutively expressed in many types of tissue and produces prostaglandins that regulate normal cellular functions. However, COX-2 activity is induced by inflammatory cytokines to produce prostaglandins that mediate the inflammatory response. Traditional nonspecific NSAlDs inhibit both COX-1 and COX-2, and in doing so, not only decrease inflammation and pain but also promote gastrointestinal tract damage and bleeding. Therefore, development of a new class of anti-inflammatory drugs that primarily inhibit COX-2 while sparing the enzymatic activity of COX-1 at therapeutic doses may offer increased safety for long-term use of NSAlDs in chronic inflammatory diseases such as RA. Celecoxib, a novel NSAlD that selectivly inhibits the enzyme COX-2, is now approved by the US Food and Drug Administration (FDA) for treatment of RA and osteoarthritis (OA). In active RA, treatment with celecoxib 100 to 400 mg twice daily and naproxen 500 mg twice daily achieved significant reductions in pain, morning stiffness, and tender/swollen joints. In patients with stable RA, celecoxib 200 mg twice daily showed sustained symptomatic improvements similar to those of twice-daily slow-release diclofenac 75 mg over a 24week period. In patients with OA of the knee, celecoxib 100 and 200 mg once and twice daily had efficacy superior to placebo and comparable to naproxen 500 mg twice daily. Celecoxib has been further indicated in patients with familial

adenomatous polyposis and/or colon cancer. It is postulated that NSAlDs may act to retard, block, or reverse colonic carcinogenesis, and celecoxib, being a COX-2 inhibitor, has the advantage of causing minimal gastrointestinal adverse effects.12 The common adverse effects reported with celecoxib are nausea, diarrhea, and abdominal pain. The incidence of upper gastrointestinal ulcer complications was eightfold lower with celecoxib than with nonspecific NSAIDs, but similar to that in patients taking placebo.Thus, the gastrointestinal tolerability of celecoxib is much better than that of traditional NSAIDS.

Pharmacokinetic studies of celecoxib in healthy subjects showed that peak blood concentrations occur 3 hours after oral administration. A high-fat meal delayed intestinal absorption of celecoxib but increased its bioavailability by -40%. Celecoxib is highly bound to plasma proteins (97%) and its volume of distribution is -400 L. The drug is metabolized predominantly in the liver via cytochrome P450 2C9, and 3% of celecoxib is excreted unchanged in urine and feces. Therefore, the drug may accumulate in the body in patients with moderate hepatic impairment but not in patients with chronic renal failure. However, elimination of the drug in patients with severe renal insufficiency has not yet been investigated. Interactions between celecoxib and other concomitantly administered drugs have been investigated. Fluconazole, a hepatic microsomal enzyme inhibitor, may cause elevation of plasma concentration of celecoxib up to twofold. Celecoxib 200 mg/d may raise the plasma concentration of lithium administered at a dosage of 450 mg twice daily if the 2 drugs are given together.” Concomitant administration of warfarin and celecoxib in healthy volunteers showed no significant effect on prothrombin time or steady-state pharmacokinetics of S- or R-warfarin.

Rofecoxib is another novel drug with a specific inhibitory effect on COX-2. It was approved by the FDA in May 1999 for the treatment of OA, primary dysmenorrhea, and acute pain in adults but has not yet received FDA approval for RA. Treatment with rofecoxib 12.5 to 25 mg/d has been found to be well tolerated. The most frequent adverse effects reported for the drug are upper respiratory tract infection, diarrhea, nausea, headache, insomnia, and edema. All the adverse effects were transient, and none required patients to discontinue therapy. Furthermore, the occurrence of gastrointestinal ulcers as common complications of traditional NSAID

therapy has been significantly reduced with rofecoxib administration to a level comparable to that of placebo. Pharmacokinetic studies showed no effect of fatty meals on the oral bioavailability of rofecoxib. Plasma protein binding was 87%. The drug is predominantly metabolized in the liver by cytosolic enzymatic reduction, with hepatic cytochrome P450 having a minor role. Renal elimination of rofecoxib is cl%, and its elimination half-life is 17 hours. The drug is not recommended for use in patients with hepatic impairment, and safety information about its use in patients with advanced renal disease is still lacking. Interactions have been reported between rofecoxib and other drugs given concomitantly. Coadministration of rofecoxib and angiotensin-converting enzyme inhibitors may cause elevation in mean arterial pressure by 3 mm Hg.

The plasma concentration of either methotrexate (MTX) or lithium may increase when rofecoxib is coadministered. The prothrombin time was increased when rofecoxib was given concomitantly with warfarin. Administration of rifampin decreased the plasma concentration of rofecoxib by 50%.22 From the pharmacoeconomic point of view, the use of COX-2 inhibitors would seem to be cost-effective, since the incidence of gastrointestinal adverse events, especially bleeding and ulcers, is reduced with these drugs. However, in a l-year assessment of the cost-effectiveness potential of COX-2 inhibitors, the reduction in gastrointestinal adverse effects did not translate into economic benefit, and more long-term studies are needed. It is encouraging that anti-inflammatory drugs such as celecoxib and rofecoxib do not have many of the side effects of glucocorticoids and traditional NSAIDs. While their long-term safety is being established by pharmacovigilance studies, they could be prescribed in the at-risk population or for other indications (eg, preterm labor, colorectal cancer). However, further investigations are needed to solve the following therapeutic problems: (1) whether these drugs are therapeutically effective at doses that affect only the desired enzymeisoforms; (2) whether near-complete inhibition of COX-2 is safe, especially if sustained for prolonged periods; (3) whether inhibition of COX-2 will prove adequate for interrupting the inflammatory response in the setting of entirely intact COX-l-dependent prostanoid production; and (4) what the long-term effects of COX-2 inhibition will be on renal function. (Ehab .2001)

DISEASE-MODIFYING ANTIRHEUMATIC DRUGS

The disease-modifying antirheumatic drugs (DMARDs) are not a new concept in the treatment of RA; several of these drugs are currently available. They include MTX, sulfasalazine (SSZ), hydroxychloroquine (HCQ), cyclosporine, gold salts, penicillamine, and azathioprine. These agents are expected to slow radiologically identifiable signs of disease progression in RA, but their long-term impact on disability has yet to be proven. It has been reported that early, combined therapy with DMARDs is more beneficial and less hazardous than late monotherapy in patients with RA. (Ehab 2001)

Methotrexate

The folic acid antagonist MTX is increasingly regarded as the agent of first choice in the treatment of RA because of its early onset of action and superior efficacy and tolerability. It is usually given orally at a dosage of 7.5 to 15 mg once weekly. Since the bioavailability of oral MTX is reduced with doses >20 mg, the drug is administered intramuscularly in that dosing range. Clinical response to MTX in RA is often observed 2 to 3 weeks from initiation of therapy, and -50% to 67% of patients continue long-term therapy with MTX.2 Gastrointestinal upset, elevation of liver enzymes, and hemocytopenia are adverse effects of MTX that may lead to treatment discontinuation. Folic acid administration in doses of 1 to 10 mg/d during therapy with MTX may reduce the gastrointestinal adverse effects of MTX without interfering with its efficacy. Pneumonitis is a rare side effect of long-term therapy with MTX. Its incidence may be higher in RA patients who are aged ~65 years and have interstitial lung disease.The adverse effects of MTX are usually observed in the first year of treatment; extended follow-up revealed no clinically detectable cumulative toxicity over a 5-year period. The efficacy of MTX when given weekly in small doses in RA is not related to the plasma concentration of the drug. It is suggested that MTX reduces the concentration of circulating purines and pyrimidines, consequently decreasing their availability for DNA and RNA synthesis. This may affect proliferation of immune cells and expression of cytokines.

Antibiotics

An infectious etiology of RA has long been postulated but has not been proved. Despite insufficient evidence for the infectious nature of this disorder, several antibacterial agents, such as sulfa compounds (e.g, SSZ) and tetracyclines (eg,minocycline) have been shown to be efficacious in the treatment of RA. SSZ is most commonly used in Europe to treat RA. The drug combines an anti-inflammatory agent (5aminosalicylic acid) and a sulfa antibiotic (sulfapyridine). The latter component is the active metabolite of the drug. The mechanism of action of SSZ against RA is unknown. It is possible that SSZ causes alteration in the intestinal flora of RA patients. Furthermore, the drug has immunologic effects in RA. SSZ is given initially at a dosage of 500 mg once or twice daily, and the dosage is gradually increased over several months to 2 to 3 g/d. This dosage regimen improves the gastric tolerance to SSZ and reduces the gastrointestinal adverse effects that may lead to drug discontinuation. Neutropenia and thrombocytopenia may occur in 10% to 25% of cases and become serious in 2% to 5% of cases. These toxicities are most likely to occur in the first 6 months of therapy; therefore, complete blood count should be monitored periodically. Minocycline 200 mg/d has been used in the treatment of RA. Recent studies demonstrated a beneficial effect of the drug in both early and advanced stages of the disease. Among patients with seropositive RA, remissions are more frequent and the need for DMARD therapy is less in those treated early in the disease with minocycline compared with patients given conventional therapy delayed by an average of only 3 months. Therefore, minocycline may be beneficial in patients with RA, especially when given early in the disease course or in patients with mild disease.In RA, minocycline may suppress T-cell activity (immunomodulatory) and inhibit metalloprotease enzymes (antiinflammatory). Both are necessary to mediate joint destruction. Dizziness is the most commonly reported side effect during minocycline therapy. Other side effects include gastrointestinal upset, rash, pneumonitis, and headache. Minocycline appears promising as a DMARD, but its use is limited by the lack of comparative studies.

Hydroxychloroquine

The antimalarial drug HCQ has been prescribed for the treatment of RA. It is usually given at 400 mg/d, but its delayed onset of action in RA (3 to 6 months) has led to the suggestion of a higher initial dosage to accelerate the clinical response. A

loading dosage of either 800 mg/d or 1200 mg/d for 6 weeks followed by a maintenance dosage of 400 mg/d has been found to hasten the response to HCQ. Unlike many other DMARDs, HCQ is not associated with renal, hepatic, or bone marrow toxicity in patients with RA. However, patients may discontinue the drug because of the most frequent gastrointestinal adverse effects (nausea, vomiting, and abdominal pain). Other side effects reported are rash, vertigo, headache and insomnia. The principal ocular adverse effect of HCQ is a progressive retinopathy involving color vision changes. This retinopathy has been noted to occur without early warning signs and, therefore, ophthalmologic examinations are mandatory before therapy is started and every 4 to 6 months thereafter. The high loading dosage of HCQ (800-1200 mg/d) has not been associated with greater ocular toxicity than the conventional dosage (400 mg/d) commonly used in RA. Furthermore, the new dosage regimen for HCQ (up to 1200 mg/d for 6 weeks) is well tolerated, except for the gastrointestinal adverse events.

Triple Therapy

Triple therapy, a successful combination therapy for early treatment of RA, consists of concomitant administration of MTX, SSZ, and HCQ. Application of this combination has been favored and has increased substantially for 3 reasons. First, most of the DMARDs lose their efficacy over time. Second, rheumatologists are not content with minor improvements in their patients. Finally, recent data indicate that combination therapy in RA can be safe and still be more efficacious than monotherapy with a DMARD. Triple therapy is advocated early in the disease, especially in patients with partial response to any of the DMARDs, SSZ plus HCQ had efficacy similar to that of MTX alone in RA. However, triple therapy (MTX + SSZ + HCQ) achieved a substantially greater benefit in patients with RA. Therefore, MTX is considered a basic drug in the Triple therapy. The adverse effects of triple therapy include gastrointestinal intolerance, weight gain, and elevation of liver enzymes. However, these side effects may be better tolerated in patients who are responding well to the triple regimen, even if the dosage has been increased up to 22.5 mg/wk (MTX) or 2 g/d (SSZ). Attempts to taper any of the 3 drugs in triple therapy may lead to disease flare.

Cyclosporine

Cyclosporine is an immunosuppressive drug used as a DMARD in the treatment of RA. In RA patients with suboptimal response to MTX alone, addition of cyclosporine can be effective. The recommended dosage of cyclosporine in combination with MTX ranges between 2.5 and 3.5 mg/kg per day but should not exceed 5 mg/kg per day. The previously recommended dosage of 10 mg/kg per day was toxic to the kidney. Cyclosporine and MTX have complementary mechanisms of action in RA. Cyclosporine inhibits interleukin-2 (IL-2) production and T-cell activity, whereas MTX inhibits IL-1 production and the activity of macrophages and monocytes. Cyclosporine is poorly absorbed orally (bioavailability is 30%), and both bile flow and ingestion of food can affect its intestinal absorption. Therefore the conventional cyclosporine (cyclosporin A) has been formulated as a microemulsion to improve its oral bioavailability. In this new formulation, cyclosporine is incorporated into a microemulsion of surfactant, ethanol, and a lipophilic and hydrophilic solution. When given orally in the micro emulsion formulation, the absorption of cyclosporine was greatly increased and its pharmacokinetic profile became more predictable. The common side effects of cyclosporine therapy are hypertension and renal impairment. They may be reversed by reducing the dose of drug or discontinuing therapy. The immunomodulating effects of cyclosporine may increase the relative risk of malignancy.Therefore, cyclosporine is not recommended for patients with RA who have any of the following conditions: current or past malignancy, except basal cell carcinoma; uncontrolled hypertension; renal dysfunction; or abnormal increase in liver enzyme activity (22 times baseline values). Cyclosporine metabolism in the liver via the cytochrome P450 enzymes may cause interactions with other coadministered drugs. Ketoconazole, erythromytin, calcium antagonists, and H, antagonists may inhibit drug metabolism, thereby elevating its plasma concentration. On the other hand, anticoagulants and rifampin increase cyclosporine metabolism and reduce its plasma concentration.

Gold Salts

Gold salts may be used in RA patients who have evidence of erosive joint lesions or who are responding unfavorably to conservative treatment. Gold salts come in oral auranofin and intramuscular preparations (gold sodium thiomalate). The usual dosage of auranofin is 3 mg twice daily, whereas injectable gold is usually given as 25

to 50 mg weekly until the patient responds. Then, the interval between injections is gradually increased to 2 to 4 weeks, according to the patient's response. The onset of action of gold in RA is usually between 2 and 6 months from the initiation of therapy. It has been reported that the addition of MTX orally at a dosage of 7.5 mg weekly to injectable gold (50 mg once weekly) for the first 6 months of treatment in patients with early RA is more beneficial than gold monotherapy. Periodic monitoring of urine for protein, as well as complete blood count and platelet count for decreases in marrow cell lines, is essential for early identification of renal or bone marrow toxicities that may be induced by gold administration. If protein urea is evident, treatment should be discontinued. Other side effects include diarrhea (auranofin) and skin rash and stomatitis (injectable gold).

Penicillamine

Penicillamine is a DMARD that is not used frequently in patients with RA because of the risk of autoimmune reactions such as systemic lupus erythematosus, polymyositis, myasthenia gravis, and Goodpasture's syndrome. Other adverse effects include proteinuria, myelosuppression, stomatitis, and rash. The initial dosage of the drug is 125 to 250 mg/d (given on an empty stomach); the dosage is gradually increased to 500 to 750 mg/d over several months to reduce the likelihood of toxicity. Clinical response to the drug is not usually expected until several months after initiating therapy. Penicillamine may induce proteinuria and myelosuppression, necessitating regular monitoring of urine for protein, as well as complete blood count and platelet count. Because of the potential adverse effects of the drug, penicillamine has become a second line treatment for RA.

Azathioprine

The purine analogue azathioprine is used in severe cases of RA. It is usually given in the range of 1 to 2 mg/kg per day. However, the high risk for myelosuppression and opportunistic infection associated with azathioprine therapy limits its use in RA. Recently azathioprine therapy has been tried in patients with active refractory RA. It was administered as a single intravenous loading dose of 22.5 to 36 mg/kg body weight followed by an oral maintenance dose of 1.7 to 2.5 mg/kg per day. Despite patient's tolerance to this dosing regimen, the loading dose did not appear to enhance control of the disease sufficiently to warrant a controlled clinical trial of this regimen.

CORTICOSTEROIDS:

Corticosteroids can provide effective symptomatic therapy in RA. Because of their multiple adverse effects, corticosteroids are now recommended in small oral doses (eg, prednisone 7.5 mg/d) that may retard progression of bone erosion in RA. Monthly pulses with high doses may hasten the response to initial DMARD therapy. Also, corticosteroids may be given chronically when the response to combination therapy with NSAlDs plus DMARDs is inadequate. Furthermore, short courses of corticosteroids may be used during acute disease flares to reduce inflammation.Compared to NSAlDs, prednisolone proved more efficacious in reducing joint tenderness and pain, whereas the difference in grip strength was insignificant. The immunomodulatory and anti-inflammatory effects of corticosteroids in RA may be achieved through multiple mechanisms. They may inhibit nuclear factor kappa B, a unique cellular transcription factor that is involved in autoimmune inflammatory reactions. Corticosteroids may also increase expression of anti-inflammatory cytokines (eg, IL-4).

Corticosteroids are associated with many adverse effects, including osteoporosis, hypertension, hyperglycemia, increased appetite, fluid retention, weight gain, central nervous system effects, cataracts, glaucoma, and progression of atherosclerosis.

Corticosteroid-induced osteoporosis has presented a challenge to clinicians for many years. Bone loss during long-term corticosteroid therapy is mediated by inhibition of gonadal and adrenal steroid production leading to hypogonadism and a direct negative effect on calcium absorption and osteoblast function. Therefore, both prevention and treatment of corticosteroid-induced osteoporosis are feasible with bisphosphonates or hormones. Bisphosphonates are considered the first option and are especially recommended for patients who meet any of the following criteria and who also use prednisolone (or an equivalent drug) 7.5 mg/d ,a history of premature menopause or a low-trauma fracture; a family history of osteoporosis; immobility; low body weight; or low bone mineral density. In all these conditions, bisphosphonates are usually indicated, especially when the patients are unwilling to take hormone replacement therapy. Hormonal therapy may be indicated in postmenopausal women and in all men and premenopausal women with evidence of

hypogonadism. Hormone replacement therapy with sex steroids increases bone mineral density in the lumbar spine in patients with corticosteroid- induced osteoporosis. It has been suggested that hormone replacement therapy not be added to bisphosphonates unless the response to bisphosphonate monotherapy is insufficient. When patients cannot tolerate bisphosphonates, or there are concerns about safety (eg, in younger patients), calcitriol is an alternative. Calcitriol can protect the spine, but not the hip joints, from osteoporosis. (Ehab .2001)

INTRODUCTION TO CYTOKINE BASED THERAPIES

Cytokines frequently have multiple biologic functions with overlapping effects. Often they have proinflammatory as well as anti-inflammatory activity,although in most cases they can be classified predominantly as proinflammatory or anti-inflammatory cytokines. The therapeutic effects of cytokine targeted therapies also depend on the type of product, dose, dosing schedule, route of administration, mechanism of action, and patient population studied. Interleukin-1P (IL-lb) and TNF-a are the key proinflammatory cytokines implicated in the pathogenesis of RA. Secreted by synovial macrophages, IL- 1 p and TNF-a stimulate synovial cells to proliferate and produce collagenase, thereby inhibiting proteoglycan synthesis, degrading cartilage, and stimulating bone resorption. These cytokines induce the expression of adhesion molecules, resulting in further inflammatory cell recruitment and release of cytokines. As a prominent mediator of the inflammatory response, TNF-a secretion stimulates release of IL-lp and IL-6 as well as other cytokines and metabolites of arachidonic acid & regulated overproduction of TNF-a in transgenic mice results in the development of a chronic polyarthritis resembling RA. A variety of proteins regulate the effect or functions of cytokines in vivo and have been recombinantly constructed as therapeutic agents designed to inhibit IL- 1 p and IL- 1 a. These include soluble-receptor antagonists and antibodies to cytokines. Soluble receptors are truncated forms of the cell surface receptor devoid of the transmembrane and intracytoplasmic domains that still retain binding affinity comparable to the full-length membrane-bound receptors. Soluble-receptor molecules bind free cytokine, inhibiting its binding to cell surface receptors. To be effective, they must be retained within the circulation. A variety of constructs have been generated by fusing the immunoglobulin G subclass (IgGl) Fc region sequences to soluble cytokine receptors, thereby increasing affinity for the cytokine and prolonging

their half-life in the circulation. In comparison, anticytokine monoclonal antibodies have much higher (lOOO-fold) affinity and can bind cell surface-bound as well as free cytokine. They are smaller, possibly enabling a wider distribution, and often have a longer circulating half-life. (Strand)

NEW DRUGS OF CYTOKINE BASED THERAPIES

Several promising new drugs have been approved by the FDA for the treatment of RA. These new medications include leflunomide, etanercept, and infliximab.

Leflunomide

Leflunomide is a new isoxazole immunomodulatory drug that acts through inhibition of de novo pyrimidine synthesis in activated T cells by selective inhibition of dihydrorotate dehydrogenase. Thus the drug may be said to have antiproliferative activity against T cells. Preliminary data suggest that leflunomide could be used not only as an alternative to MTX as first-line treatment in RA but also in combination with MTX in patients who are unresponsive to MTX monotherapy. Leflunomide has the advantages of rapid onset of action (-4 weeks after the initiation of therapy) and significant slowing of disease progression, demonstrated by radiographic findings. Therefore, leflunomide may be considered a DMARD. Leflunomide is a prodrug given orally and metabolized in the intestine and liver to an active form that reaches its peak level in plasma within 8 to 12 hours. The active drug is metabolized further and excreted in urine and feces with an elimination half-life of 2 weeks. It is usually given in FU at a loading dosage of 100 mg/d for 3 days followed by a maintenance dosage of 20 mg/d. The adverse effects of the drug include reversible alopecia, diarrhea, elevated hepatic aminotransferase activity, and weight loss. The drug is considered teratogenic in animals and, therefore, should not be used by pregnant women. Interactions have been reported between leflunomide and cholestyramine. The latter drug binds to leflunomide in the intestine, decreasing its absorption. Furthermore, cholestyramine increases the rate of clearance of leflunomide from the body. Therefore, cholestyramine may be used in case of leflunomide toxicity.

Etanercept

The biologic agent etanercept is a recombinant version of soluble human tumor necrosis factor receptor (sTNFR) approved by the FDA for the treatment of RA. Tumor necrosis factor (TNF) is one of the major cytokines responsible for induction of inflammation in RA. In the synovium of the joint, TNF binds to cell surface receptors p55 and p75, stimulating synoviocyte proliferation and production of inflammatory mediators. This leads to recruitment of inflammatory cells and joint destruction. The sTNFRs, which are regulatory receptors to TNF inflammatory activity, are described as truncated versions of membrane TNF receptors. The role of sTNFRs is to prevent TNF access to p55 and p75 receptors, thus inhibiting the inflammatory activity of TNF. In RA, sTNFRs are expected to be reduced in number, allowing extensive binding of TNF to their own receptors (~55 and ~75) with consequent induction of inflammation. Etanercept is a competitive inhibitor of the binding of TNF to p55 and p75 receptors. If used in RA, it is usually given alone or in combination with MTX in patients who are not responding to MTX monotherapy or therapy with other DMARDs. In patients with RA who failed to improve with DMARD, subcutaneous administration of etanercept 16 mg/m twice weekly or as a fixed dose of 25 mg/wk achieved a clinical response rate of 20% as defined by the American College of Rheumatology (ACR). In RA patients who did not respond to MTX after 6 months, addition of etanercept 15 to 25 mg/wk achieved a clinical response of 220% as defined by the ACR. Discontinuing etanercept monotherapy in RA leads to recurrence of the disease symptoms within month after drug withdrawal. Etanercept is usually given by subcutaneous injection 25 mg twice weekly. The drug is generally well tolerated, but it can produce local inflammation at the site of injection. Patients with life-threatening infection should discontinue etanercept therapy.

lnfliximab

Infliximab is a chimeric human/mouse monoclonal antibody approved by the FDA for treatment of Crohn’s disease and RA. It binds to TNF-alpha and neutralizes it. Administration of the drug as a single intravenous infusion of 10 mg/kg body weight in patients with RA who failed treatment with DMARD resulted in significantly greater clinical improvement compared with placebo. The duration of response to the drug, which varied from 1 to 19 weeks, appeared to decrease with

repeated infusions. In patients with RA refractory to MTX, the combination of infliximab and MTX was found to be more effective than either of the 2 drugs alone. These 2 drugs should be administered concomitantly to guard against development of antibodies against infliximab, since these antibodies may limit both the duration of action and maximal response to the drug. Reported adverse effects of infliximab therapy include hypersensitivity reactions (eg, fever, chills, urticaria, dyspnea, and hypotension), lupus-like reactions, and increased incidence of infection. Etanercept and infliximab treatment should be reserved for patients who fail to respond to DMARD therapy. They may be given either alone or in combination with MTX. However, further studies should be conducted to assess the long-term safety and efficacy of these new agents as well as their role in combination therapy for RA. (Strand)

EXPERIMENTAL THERAPIES

Research on the treatment of RA continues, and the efficacy of other new agents is being investigated. Among the therapies currently being assessed are biologic agents, gene therapy, vitamin E, and stem cell transplantation.

Anti-CD4 Monoclonal Antibodies

Anti-CD4 monoclonal antibodies (anti-CD4MAb) are biologic agents directed against CD4 T cells. These cells are involved in the initiation of RA through promotion of humoral and cell-mediated immune responses. Anti-CD4MAb may exert an effect via several mechanisms: immunosuppression by depletion of T cells, inhibition of class II major histocompatibility complex-dependent T-cell responses by blocking the interaction of CD4 T cells with antigen presenting cells, or transmission of an inhibitory signal to the CD4+ cells. Because of the immunogenic reactions induced by murine anti-CDCMAb initially assessed as therapeutic agents in RA, chimeric and humanized MAb are now used in clinical trials. They are less immunogenic. Early studies of chimerit anti-CD4MAb therapy in patients with RA resulted in marked and prolonged reduction of circulating CD4 T cells without a parallel reduction in synovial tissue CD4 T cells. Therefore, insufficient clinical benefit associated with the high risk of fatal infection was reported in these trials. The biotechnology industry has created increasingly humanized MAb with the hope of minimizing immunogenicity, inhibiting functions of relevant T cells, and minimizing

depletion of circulating T cells. They are called nondepleting humanized anti-CD4-MAb and are now being studied as treatment for RA. The preliminary results are encouraging.

Interleukin-1 Receptor Antagonist

IL-l receptor antagonist (IL-lra), a biologic agent, is an anti-inflammatory cytokine produced primarily by activated monocytes and tissue macrophages. In RA the concentrations of IL-l are increased in the synovial fluid of affected joints. However, this increase may be insufficient to inhibit the IL-l-induced inflammatory response occurring in RA. Administration of IL-l at a dosage of 150 mg/d in patients with RA achieved a modest inhibition in both joint swelling and radiographic evidence of progression of bone lesions. No side effects were reported, except transient injection-site reactions. IL-l is the first biologic agent to demonstrate a significant reduction in the rate of joint damage in patients with RA. Therefore, it is considered an important novel therapeutic option for future management of RA.

Interleukin-10

IL-10 is a natural anti-inflammatory cytokine produced mainly by monocytes. Its physiologic functions include inhibition of proinflammatory cytokines (IL-1 and TNF) and antigen-presenting cell function.IL-10 expression in the synovial fluid and serum increases in patients with RA, where it may serve as a buffer or natural inhibitor of the activities of several proinflammatory cytokines. However, an insufficient amount of IL-10 is produced to effectively suppress the inflammatory response. A multicenter, randomized, double-blind, placebo controlled study investigated the use of recombinant human IL-10 in active IL-10 5 mg/kg per day was administered subcutaneously for 28 days. Trends toward clinical improvement of 220% as defined by the ACRs were observed in patients treated with IL-10 compared with placebo. Further more, DMARD usage during the 2 months following therapy was reduced in IL-10 treated patients versus placebo patients. It is said that IL-10 administration in RA may produce a reparative effect within the diseased joints through stimulation of synthesis of proteoglycan, which is necessary for regeneration of damaged joints and cartilage.

Gene Therapy

Gene therapy, the transfer of genes to patients for therapeutic purposes, is being used in the treatment of RA for a number of reasons. First, current steroidal and nonsteroidal treatments have limited efficacy and a high incidence of side effects. Second, biologic agents must be administered subcutaneously or intravenously at least once weekly, and disease symptoms return when treatment is stopped. Furthermore, susceptibility to infection may increase when using immunomodulatory molecules such as TNF and IL-l systemically in RA. Finally, local injection of biologic agents into joint spaces is followed by rapid clearance of these recombinant proteins from the synovial fluid. In this condition, daily injections may be indicated. When a gene that encodes therapeutic proteins is administered into the affected joint, it enables the joint itself to produce high local levels of these therapeutic mediators that can subsequently inhibit inflammation, synovial proliferation, and cartilage destruction. In animal models with collagen-induced arthritis, injection of antiarthritic genes into single arthritic joints could produce an antiarthritic response in uninjected joints of the same animal. This observation suggested that transduced cells traffic from injected to uninjected joints. Accordingly, there is no need to inject diseased joints individually when applying gene therapy in polyarticular diseases such as RA. The first clinical trial of gene therapy in patients with RA was initiated in 1996 at the University of Pittsburgh. In this trial, a retroviral vector carried the gene for human IL-l. The vector was injected into 2 of the 4 metacarpal joints of the right hand with the other 2 joints receiving unmodified cells. One week later, the joints were removed and the recovered tissues were analyzed for gene expression and changes in pathophysiology. In the 7 patients who completed the trial, gene expression was detected in all of the appropriate joints with no apparent toxicity. The rate-limiting step to successful application of gene therapy in RA is the ability to deliver genes efficiently and repeatedly to joints without inducing pathology. It is not yet likely that gene therapy will be routine treatment for RA. (Ehab .2001)

Antioxidants

In the last few years, antioxidants, particularly vitamin E, have received considerable attention in the treatment of human diseases. In rheumatic diseases, active phagocytes and leukocytes migrate into synovial and periarticular tissues causing liberation of active oxygen species that exacerbate and perpetuate the

rheumatoid condition. Thus in rheumatology, treatment with antioxidants has received much interest. A double blind randomized study investigated the possible analgesic and anti-inflammatory properties of orally administered tocopherol (vitamin E) at a dosage of 600 mg twice daily compared with placebo for 12 weeks in patients with RA. The study showed that vitamin E was effective in reducing pain but had no anti-inflammatory effect. In another study, vitamin E (1200 mg/d) was compared to diclofenac sodium for 3 weeks in patients with chronic RA. The study demonstrated similar therapeutic efficacy of both drugs with respect to pain relief and duration of morning stiffness.

The risk profile of NSAIDs in long-term treatment of chronic RA makes administration of high-dose vitamin E a possible alternative in the treatment of inflammatory rheumatoid diseases. (Strand)

CHAPTER - II

Review of literature

REVIEW OF LITERATURE

1) Rajesh Kumar, et al., (2009) has conducted the hepatoprotective activity of aerial parts of *Plumbago zeylanica* against Carbon tetra chloride – induced hepatotoxicity in rats. Silymarin (100mg/kg, p.o.) was given as reference drug. The extract of aerial parts of *Plumbago zeylanica* have been reported to have significant hepatoprotection against CCl_4-induced hepatotoxicity in wistar rats by reducing serum total bilirubin, SGPT ,SGOT and ALP levels. Histopathological studies also confirmed the hepatoprotective nature of the extract.[10]

2) Maryamzahin, et al., (2009) has studied the in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. Methanolic extracts of *Plumbago zeylanica* (Root), *Acorus calamus* (Rhizome), *Hemidesmus indicus* (Stem) and *Holarrhena antidysenterica* (Bark), were evaluated for their antioxidant activity by ferric thiocyanate (FTC) assay and compared with thiobarbituric acid (TBA) method. Further, the radical-scavenging activity of the extracts was measured as decolorizing activity followed by the trapping of the unpaired electron of DPPH. The findings indicated promising antioxidant activity of crude extracts of the above plants.[11]

3) Vineet Mittal, et al., (2010) had conducted a comparative study of analgesic activity of *Plumbago zeylanica* callus and root extracts in experimental mice. The activities were evaluated for peripheral and central analgesic activity by glacial acetic acid induced writhing and tail immersion model respectively. In conclusion, a massive light creamish brown and granular callus formed with MS medium supplemented with naphthalene acetic acid (1.5 ppm) and kinetin (0.25 ppm) and it possess a significant peripheral analgesic activity.[12]

4) Vishal Gupta, et al., (2011) has evaluate the wound healing activity of herbal drug combination of *Rubia cordifolia, Centella asiatica, Terminalia belerica, Plumbago zeylanica* and *Withania somnifera* on excision wound of albino rats. The activity of the plant was evaluated by formulating the drug in ointment dosage form and then compared with a marketed formulation (Soframycin

cream) as reference drug. The parameters studied include the percentage closure of excision wound, period of epithelization.[13]

5) Kanchana, N, et al., (2009) has evaluated the hepatoprotective effect of *plumbago zeylanica* on paracetmol induced liver toxicity in rats. In serum total bilirubin, total protein, aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, γ-Glutamyl transferase, Total Cholesterol and serum triglycerides were determined to assess the effect of the extract on the paracetamol induced hepatic damage. The study was also supported by histopathology of liver sections.[14]

6) Sasi kumar, et al., (2010) has conducted the HPTLC analysis of various market samples of a traditional drug source –Kodiveli (*Plumbago zeylanica*). The present study deals with Plumbagin, 5-hydroxy-2methyl-1, 4-napthoquinone, an active chemical constituent of the root of this plant drug. This bioactive molecule is a proven anticarcinogenic, antiatherosclerotic and antimicrobial agent.[15]

7) Jyoti Ranjan Rout, et al., (2010) has evaluated the In Vivo Protein Profiling and Catalase Activity of *Plumbago zeylanica.* Traditionally it is used as carminative, anthelmintic, to cure inflammation, piles etc. In the first part of our study, total protein content (11.1±2.6mg/ gm of fresh leaf) was estimated from leaf sample of *P. zeylanica* in the second part, the catalase activity was studied from the leaf sample. In this study it was found that *P. zeylanica* contained several protein bands of molecular weight 50.08, 41.25, 38.41, 36.21, 28.74 and 25.52kDa and the total catalase activity was 168.40±7.28 nKat/min/mg of protein.[16]

8) Barasa M Maniafu, et al., **(2009) has studied the** larvicidal activity of extracts from three *Plumbago* species against Anopheles gambiae. The crude extracts exhibiting the highest larvicidal activity against Anopheles gambiae were hexane (LC*50* = 6.4 μg/mL) and chloroform (LC*50* = 6.7 μg/mL) extracts from *Plumbago zeylanica* Linn.[17]

9) Lie-Chwen Lin, et al., (2003) has studied the Cytotoxic naphthoquinones and plumbagic acid glucosides from *Plumbago zeylanica.* Two plumbagic acid glucosides, 30-O-b-glucopyranosyl plumbagic acid and 30-O-b-glucopyranosyl plumbagic acid methylester along with five naphthoquinones and five coumarins were isolated from the roots of *Plumbago zeylanica*. Cytotoxicity of these compounds to various tumor cells lines was evaluated, and plumbagin significantly suppressed growth of Raji, Calu-1, HeLa, and Wish tumor cell lines.[18]

10) Navneet Kishore, et al., (2010) has isolate Difuranonaphthoquinones from *Plumbago zeylanica* roots. The naphthoquinones, lapachol (1), plumbagin (2), 2-isopropenyl-9-methoxy-1,8-di-oxa-dicyclopenta[b,g]naphthalene-4,10-dione (3), 9-hydroxy-2-isopropenyl-1,8-dioxa-dicyclopenta[b,g]naphthalene- 4,10-dione (4), 2-(1-hydroxy-1-methyl-ethyl)-9-methoxy-1,8-dioxa-dicyclopenta[b,g]naphthalene- 4,10-dione (5) and 5,7-dihydroxy-8-methoxy-2-methyl-1,4-naphthoquinone (6) were isolated isolated from roots of *Plumbago zeylanica.* The new constituents in addition to known compounds were characterized by spectral analysis (UV, IR, 1D & 2D NMR and MS).[19]

11) Vijayakumar, R, et al., (2006) has evaluated the *Plumbago zeylanica* action on blood coagulation profile with and without blood volume reduction .The structure of its active principle is similar to that of vitamin K. The individual groups were screened for its effect on bleeding time (BT), clotting time (CT), prothrombin time (PT), platelet count and platelet adhesion in albino rats after 1-day, 15-day and 31-day treatment. There was no change in the platelet count in the treated groups when compared to the control levels. But the platelet adhesion was significantly decreased after PZ and also napthaquinone-treated animals in both with and without blood volume reduction after 15th as well as 31st day.[20]

12) Kefale teshome, et al., (2008) has done the toxicity studies on dermal application of plant extract of *Plumbago zeylanica.* To this effect, the dermatotoxicity of 80% methanol extract of the root part of *Plumbago zeylanica* was investigated in animals following standard procedures for irritation, sensitization, acute toxicity and repeated toxicity tests. Extraction of plant material with 80% methanol resulted in 9.45% of crude extract of *Plumbago zeylanica.* Taken together, the dermatotoxicity test results from this study suggest that *Plumbago zeylanica* toxic effects might be limited to effects like moderate irritation.[21]

13) Ravikumar , et al., (2011) has studied petroleum ether ,ethanol and aqueous extract of leaves and stems of *plumbago zeylanica* were used against bacteria and fungi by paper disc method . Gentamycin and Amphotericin – B were used as standard drug. The result suggest that ethanol and petroleum ether extract showed moderate anti- bacterial activity and aqueous extract did not active against negative bacterial species. In antifungal , it was found that among the extracts and compare with standard , ethanol extract showed a significant anti-fungal activity.[22]

14) Aparanji poosarla ,et al.,(2010) has investigated the potential immunosuppressive property by testing the aqueous extracts of the *plumbago zeylanica* parts by inducing ovalbumin specific IgG antibody responses in murine system. The Aqueous root extract of *plumbago zeylanica* exhibited the significant suppression OVA- specific IgG antibody response determined by enzyme-linked immunosorbent assay (ELISA).[23]

15) Vineet Mittal , et al.,(2010) has investigated the effect of *plumbago zeylanica* roots on learning and memory of mice. The study was carried out in extroceptive and introceptive behaviour model . The chloroform extract of *plumbago zeylanica* (100,200 and 400 mg/kg) Was administered for 10 successive days in separate group of animals. This shows promising memory enhancing effect.[24]

16) Sachin Hiradeve, et al .,(2009) has evaluvated the ethanolic extract of *plumbago zeylanica* Linn. Possessed significant anticancer activity against Ehrlich ascites carcinoma in animal model. This is due to the leaf extract contains higher amount of terpenoid and flavanoid content .[25]

17) Vishnukanta, et al.,(2010) has investigated leaf extract *of plumbago zeylanica* shows anticonvulsant activity in mice and rat using PTZ induced convulsion and maximum electro shock induced convulsion.[26]

18) Devender Rao Kodati , et al., (2011) has evaluated methanolic root extract of *plumbago zeylanica* are having significant wound healing activity in rats.[27]

19) R Jeychandran , et al., (2009) has studied plumbagin and chloroform extract of *plumbago zeylanica* L. Root showed antibacterial activity against Escherichia coli, salmonella typhi and staphylococus aureus. The methanolic extract exhibited moderate activity and aqueous extract weak activity against the bacterial strains as assessed by disc diffusion assays.[28]

20) Kantha Arunachalam , et al.,(2010) has conducted a comparitative study of Anti-inflammatory andcytotoxic effects of plumbago zeylanica. The root of plumbago zeylanica extracted with methanol was used for determining anti- inflammatory effects .In carrageen in induced raw paw oedema confirming that plumbago zeylanica roots are effective against acute inflammation . For evaluation of cytotoxicity, the crude dichloromethane extract was subjected to silica gel column chromatography . Their structure were elucidated with the help of spectroscopic techniques.[29]

21) Chen- Anchen, et al. , (2008) has evaluvated the plumbagin isolated from *plumbago zeylanica* shows anticancer or anti tumour effect against pancreatic cancer.[30]

22) Ajayi , et al . , (2011) has studided the phytochemical component present in the ethanolic root extract of the plant by phytochemical screening methods and GC-MS Analysis. The phytochemical screening showed the presence of alkaloids, tannins, Steroids, flavanoids, saponins , anthraquinones, cardiac- glycosides, phlobatinnins And carbohydrates. The GC- MS analysis also identified the presence of phytochemical components like phenolics, cyclopentadecane, fatty acid, Feroprone etc.[31]

23) Marym zahin , et al., *(*2009) has conducted the in vitro antioxidant activity and total phenolic content of four medicinal plants the present study deals with methanolic extract of *plumbago zeylanica* (root) , *Acoras calamus* (Rhizome) , *Hemidesmus* indicus (stem) and *Holarrhena antidysentrica* (Bark) evaluvated for their antioxidant activity by ferric thiocynate (FTC) assay and thiobarbituric acid (TBA) method.[32]

24) Bharathi raja subramaniya , et al., (2000) has investigated the anti proliferative and apoptotic activity of plumbagin by using two human colonic cancer cell lines ,HT29 and HCT 15.[33]

25) Alpanaram, et al. , (1996) has evaluvated the effect of ethanolic extract of *plumbago zeylanic* and combined with vitamin E was studied in experimentally induced hyperlipidemic rabbit. There was significant reduction in serum total cholesterol , LDL , cholesterol and triglycerides level.[34].

26) Meena , et al ., (2009) has conducted root of *plumbago zeylanica* was subjected to macro-microscopic, physic- chemical, preliminary photochemical, TLC to fix the quality of standard drug. This experiment yielded as set of diagnostic characters like cork cell, stone cell, parenchyma. This study would be useful for standardization of this raw drug derived from root of *plumbago zeylanica.*[35]

27) Chellampillai bothiraja, et al ., (2011) has studied the roots of *P. zeylanica* were cold macerated with a mixture of chloroform/dichloromethane (1:1) and the extract was s washed with water, saturated sodium bicarbonate and water . The concentrated washed extract was dissolved in n-hexane and recrystallized to get plumbagin. The isolated and standard plumb gin was subjected to DSC, HPTLC, UV, FTIR, 1H and 13C NMR and mass spectroscopy studies for assessment of chemical structure and purity.[36]

28) Kamal, et al., (1982) has evaluated isolation of plumbagin , droserone, isoshinanolone and a new naphthalenone, 1,2(3)-tetrahydro-3,3'-biplumbagm is reported from the phenolic fraction of the high petrol extract of the roots of *Plumbago zeylanzca*. The structure of the new naphthalenone was elucidated by means of spectroscopic data and chemical conversions. The main constituent of the neutral fraction was shown to be sitosterol.[37]

29) Akella , et al . , (1975) has conducted the isolation of six pigment from the and *plumbago zeylanica* identification of three of these as plumbagin (2-methyl-5hydroxy-1,4-naphthaquinone, PZ 4), 3chloroplumbagin(PZ 3) and 3,3'-biplumbagin (PZ 6) . From the Present study a new biplumbagin . It has been named chitranone, is a juglone derivative from its colour reactions, UV and IR spectra.[38]

30) Kefele Teshome , et al. , (2008) has studied *Plumbago zeylanica*, a medicinal plant commonly for skin diseases was subjected to asymtomatic dermatotoxicity study . To this effect, the dermatotoxicity of 80% methanol extract of the root part of *Plumbago zeylanica* was investigated in animals following standard procedures for irritation, sensitization, acute toxicity and repeated toxicity tests. Extraction of plant material with 80% methanol resulted in 9.45% of crude extract of *Plumbago zeylanica* . The skin irritation test on rabbits *Plumbago zeylanica* extract to be a moderate irritant, with a primary irritation index of 2.00. Sensitization test on mice by the Mouse Ear Swelling Test method revealed the extract to be non-sensitizer in a dose range of 4–10 mg/ml and the percent responder was zero. Acute dermal toxicity test

on rats did not produce any overt signs of toxicity, except that there was a weight gain difference between the test and control groups of female rats.The dermatotoxicity test results from this study suggest that *Plumbago zeylanica* toxic effects might be limited to effects like moderate irritation[39]

31) Gupta sandeep, et al. , (2011) has evaluated the effect of plumbagin-free alcohol extract (PFAE) of *Plumbago zeylanica* root, on female reproductive system and fertility of Adult female wistar rats . All findings suggest that the anti fertility activity of extract could possibly be through the change in the implantation site , altered hormonal level , prolonged estrous cycle and anti- estrogenic activity. Hence the extract possesses reversible anti fertility activity without adverse toxicity in females.[40]

32) Kamal , et al . , (2004) has reported the synthesis of plumbazeylanone, a quinine From Plumbago zeylanica is probably 5b,11a, 12, 12a tetrahydro-1,7 dihydroxy -5b-(-8-hydroxy-3-methyl-1,4-naphtoquinone-2-yl)- 5a,12a-dimethyl-5aH-dibenzo[b,h] fluorine-5,13:6,11-diquinone , a novel trimer of plumbagin with additional methyl group [41]

33) Sathya Shanmugaraja , et al ., (2010) has studied , the anti-invasive activities of *P. zeylanica* methanolic extract (PME) and pure compound 3_-hydroxylup-20(29)-ene-27,28-dioic acid (PZP) isolated from it are investigated in vitro. PME and PZP were noted to have the ability to induce apoptosis as assessed by flow cytometry. Further, the molecular mechanism of apoptosis induced by PME and PZP was found by the loss of mitochondrial membrane potential with the down regulation of Bcl-2, increased expression of Bad, release of cytochrome c, activation of caspase-3 and cleavage of PARP leading to DNA fragmentation Importantly, both PME and PZP were observed to suppress MDA-MB-231 cells adhesion to the fibronectin-coated substrate and also inhibited the wound healing migration and invasion of MDA-MB-231 cells through the reconstituted extracellular matrix. Gelatin zymography revealed that PME and PZP decreased the secretion of matrix metalloproteinases-2 (MMP-2) and metalloproteinases-9 (MMP-9).Interestingly both PME and PZP

exerted an inhibitory effect on the protein levels of p-PI3K, p-Akt, p-JNK,p-ERK1/2, MMP-2, MMP-9, VEGF and HIF-1_ that are consistent with the observed anti-metastatic effect[42]

34) Iwao Okamoto, et al., reported the direct biomimetic synthesis of 3, 3%-biplumbagin, isolated from *Plumbago zeylanica* 2,2%-binaphthols was developed, utilizing aryl–aryl coupling reaction via electron donor–acceptor complexes of 1-naphthols with $SnCl_4$. Heating of the complex in a sealed tube afforded the corresponding *o-o* coupling product in excellent yield. This method was utilized for a biomimetic synthesis of the binaphthoquinone,3 ,3%-biplumbagin, isolated from *Plumbago zeylanica* . *[43]*

35) Gebre-Mariam , et al., (2005) has evaluated antiviral activity of some herbal Drug combination of *Acokanthera schimperi* (Apocynaceae), *Euclea schimperi* (Ebenaceae), *Inula confertiflora* (Asteraceae), *Melilotus elegans* (Leguminosae), and *Plumbago zeylanica* (Plumbaginaceae) the antiviral activities of the 80% methanolic extracts of these plants have been examined against coxsackie virus B3 (CVB3), influenza A virus and herpes simplex virus type1 Kupka (HSV-1) using cytopathic effect (CPE) inhibitory assays in HeLa, MDCK, and GMK cells, respectively. [44]

36) Demma, et al., (2009) has conducted potential genotoxicity of herbal drug Combinations hydroalcoholic extracts of *Glinus lotoide, Plumbago zeylanica*, *Rumex steudelii* and *Thymus schimperi*were were evaluated for their DNA damaging effects using the comet assay in Mouse lymphoma L5178Y cells In the absence of S9, all extracts were found to induce significant DNA damage without affect in the cell viability. *T. schimperi* and *R. steudelii*were the most potent DNA-damaging extracts, and *G. lotoides*and *P. zeylanica* the least potent. The addition of S9 had different effects on the DNA damage induced by the extracts: It lowered the DNA damaging effect of *P. zeylanica*, did not affect the DNA damaging effectof *T. schimperi*, and increased the DNA damaging effects of *R. steudelii* and *G. lotoides* The findings of the present study suggest that all extracts evaluated have a genotoxic potential in vitro. [45]

37) *Seiichi Sakamoto,* et al., (2008) has investigated Development of an enzyme-linked immunosorbent assay(ELISA) using highly-specific monoclonal antibodies against plumbagin . plumbagin (PL; 5-hydroxy-2-methyl-1,4-naphthoquinone) is a natural compound mainly isolated from *Plumbago zeylanica*. Plumbagin has been shown to have various pharmacological activities. Assay (ELISA) system for determination of PL. 3-(5-Hydroxy-2-methyl-1,4-naphthoquinone-3-yl) propanoic acid was synthesized and purified to prepare PL–bovine serum albumin conjugate (PL–BSA), which was used as an immunogen. PL–BSA conjugate was administered into BALB/c male assay (ELISA) system for mice for production of monoclonal antibodies against PL. The monoclonal antibody against PL which is secreted from established hybridoma cell line 3A3 (MAb 3A3) has been proven to have highly-specific to PL resulting from cross- reactivity test. The range for calibration of PL by ELISA was 0.2–25_gmL−1. Based on validation analysis, this analytical method by ELISA is a precise, accurate, and sensitive method for the determination of PL in plant . [46]

38) Yen-Ju Hsieh, et al., (2006) finds to develop an automated blood sampling (ABS) method coupled to a liquid chromatography–tandem mass spectroscopy (LC–MS/MS) method to evaluate the oral bioavailability of plumbagin in a conscious freely moving rat. Plumbagin, an herbal ingredient, was isolated from *Plumbago zeylanica*. The separation was performed using a reversed phase C18 (150mm×4.6mm I.D.; 5_m) column and was eluted with the mobile phase of water–acetonitrile (40:60, v/v) at a flow-rate of 0.8 ml/min. Multiple reaction monitoring (MRM) was used to monitor the transition of the deprotonated molecule *m*/*z* 187 [M H] to the product ion *m*/*z* 159 [M H CO]− for the plumbagin analysis. The calibration curve was linear over the concentration range of 10–2000 ng/ml with a coefficient estimation of 0.995. The intra- and inter-day variations (% relative standard deviation; RSD and % bias) of the assay for rat plasma samples were less than 17%. The limit of detection and the limit of quantification were 5 and 10 ng/ml, respectively. Recovery of plumbagin from the rat plasma was about 80%. This LCMS/MS method has been validated to study the pharmacokinetics plumbagin was about 38.7±5%. [47]

39) Akella , et al., (1978) has studied The isolation of Zeylanone And Isozeylanone two novel quinines , from *plumbago zeylanica* . [48]

40) Lie-Chwen Linn, et al., (2002) has isolated two plumbagic acid glucosides, 30-O-b-glucopyranosyl plumbagic acid and 30-O-b-glucopyranosyl plumbagic acid methylester along with five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), and five coumarins (seselin,5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin) were isolated from the roots of *Plumbago zeylanica* and Cytotoxicity of these compounds to various tumor cells lines was evaluated. [49]

41) Yen-Ju Hsieh, et al., (2005) has studied liquid chromatography coupled with tandem mass spectrometric (LC–MS/MS) method for the determination of plumbagin form *plumbago zeylanica.* [50]

42) Yuan-Chuen Wan. , et al., (2005) has investigated plumbagin's anti-*H. pylori* activityand developed a reversed-phase high-performance liquid chromatography (HPLC) method for quantification of plumbagin from *P. zeylanica* L. And observed that plumbagin has strong anti-*H.Pylori* activity, with 0.02–0.16 mg/ml as minimum inhibitory concentrations and 0.16–1.28 mg/ml as minimum bactericidal concentrations. [51]

43) Kamal .Gunaherath, et al., (1982) has investigated a method to develop plumbagm, droserone, isoshinanolone and a new naphthalenone, 1,2(3)-tetrahydro-3,3'-biplumbagm is reported from the phenolic fraction of the light petrol extract of the roots of Plumbago zeylanzca. The structure of the new naphthalenone was elucidated by means of spectroscopic data and chemical interter conversions. The main constituent of the neutral fraction was shown to be sitosterol . [52]

44) Navneet Kishore ,et al., (2009) has examined the isolation of Difuranonaphthoquinones such as naphthoquinones, lapachol (1), plumbagin (2), 2-isopropenyl-9-methoxy-1,8-di-oxa-dicyclopenta[b,g]naphthalene-4,10-dione(3),9-hydroxy-2-isopropenyl-1,8-dioxadicyclopenta[b,g]naphthalene-

4,10-dione (4), 2-(1-hydroxy-1-methyl-ethyl)-9-methoxy-1,8-dioxa-dicyclopenta[b,g]naphthalene-4,10-dione (5) and 5,7-dihydroxy-8-methoxy-2-methyl-1,4-naphthoquinone (6)from roots of *Plumbago zeylanica* .[53]

45) Yuan-Chuen et al., (2004) has evaluated Anti-Helicobacter pylori activity of *Plumbago zeylanica*. [54]

46) Tsai, et al., (2008) has studied Seselin from *Plumbago zeylanica* inhibits phytohemagglutinin (PHA)-stimulated cell proliferation in human peripheral blood mononuclear cells. [55]

47) Praveen Verm , et al ., (2001) has evaluated rapid micro propagation and establishment of higher plumbagin yielding hairy root cultures by using in vitro - studies in plumbago zeylanica. Hairy roots initiated at 0.9 ± 0.05 relative transformation frequency with the A_4 strain of *Agrobacterium rhizogenes* exhibited optimum growth in half strength MS medium containing 4% sucrose. Growth kinetic studies demonstrated a maximum 21 fold increase in biomass yield after 6 weeks of culture. The fresh hairy roots produced 2.5 times higher amounts of plumbagin than the fresh, untransformed control roots or the dry hairy roots of the same age. The present research finding srevealed for the first time the potentialities of the hairy root cultures of *Plumbago zeylanica* for the production of the important secondary metabolite plumbagin. [56]

48) Yen-Ju Hsieh , et al. , (2005) has evaluated liquid chromatography coupled with tandem mass spectrometric (LC–MS/MS) method for the Determination and identification of plumbagin form root of *plumbago zeylanica* . [57]

49) Navneet Kishore , et al ., (2009) has studied the isolation naphthoquinones, lapach(1),plumbagin(2)2-isopropenyl-9-methoxy-1,8-di-oxdicyclopenta[b,g]naphthalene-4,10-dione (3), 9-hydroxy-2-isopropenyl-1,8-dioxa-dicyclopenta[b,g]naphthalene-4,10-dion(4),2-(1-hydroxy-1-methyl-ethyl)-9-methoxy-1,8-dioxdicyclopenta[b,g]naphthalene-4,10-dione (5) and 5,7-dihydroxy-8-methoxy-2-methyl-1,4-naphthoquinone (6) from roots of

Plumbago zeylanica .The new constituents (3–5) in addition to known compounds (1, 2 and6) were characterized by spectral analysis (UV, IR, 1D & 2D NMR and MS). [58]

50) Shanumugasundaram P, et al. , (2006) has evaluated hepato protective and antioxidant effects of *hydrophila auriculata* root against ccl_4 induced liver toxicity in rats and ferric thiocyanate (FTC) and thiobarbituric acid (TBA) method respectively . The activity was accessed by monitoring the various liver function tests , viz alanine transaminase , aspartate transaminase (AST) , alkaline phosphatase (ALP) , Total protein and total bilirubin. Furthermore hepatic tissues were subjected to histopathological studies. They concluded that there was a significant hepatoprotective activity and antioxidant activity for aqueous extract of the roots of *hygrophilia auriculata* . [59]

51) Rawat , et al ., (1997) studided hepatoprotective activity of *Boerhaavia diffusa* L.roots a popular indian ethanomedicine . In this study the effects of seasons, thickness of roots and form of dose were studied for their hepatoprotective action to prove the claims made by the different diameters tribes of india. The hepatoprotective activity of roots of different diameters collected in three seasons , rainy, summer, and winter , was examined in thioacetamide intoxicated rats . The result showed that an aqueous extract (2ml/kg) of roots of diameter 1-3 cm , collected in this month may (summer) exhibited marked protection of a majority of serum parameters , ie. S GOT, SGPT. [60]

52) Anubha singh , et al . , (1995) has evaluated the hepatoprotective activity of *Apium gaveolens* and *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats . In this study the rat liver was damaged by a single dose of paracetamol (3kg/kg:p.o) or thioacetamide (100mg/kg; s.c). They were monitoring several liver function tests, Viz. serum transaminase (SGOT and SGPT) , alkaline phosphatase, sorbitol dehydrogenase , glutamate dehydrogenase, and bilirubin in serum . They concluded that there was a significant hepatoprotective activity of the

methanolic extract of the seeds of *Apium Graveolens* and *Hygrophila auriculata*. [61]

53) Muthu Gounder Palanivel, et al., (2008) has studied Ethanol extract of Pisonia aculeata (EPA) was evaluated for hepatoprotective and antioxidant activities in rats. The plant extract (250 and 500 mg/kg, p.o.) showed a remarkable hepatoprotective and antioxidant activity against carbon tetrachloride (CCl_4)-induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. CCl_4-induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST). Treatment of rats with different doses of plant extract (250 and 500 mg/kg) significantly ($P<0.001$) altered serum marker enzymes and antioxidant levels to near normal against CCl4-treated rats. The activity of the extract at dose of 500 mg/kg was comparable to the standard drug, silymarin (50 mg/kg, p.o.). Histopathological changes of liver sample were compared with respective control. Results indicate the hepatoprotective and antioxidant properties of *P. aculeata* against CCl_4-induced hepatotoxicity in rats . [62]

54) Deepak. k. Das, et al., (2007) has studied the hepatoprotective effect of chloroform and methanol extract (CEIF and MEIF) of whole plant of *I. frutescens* (Linn.) by paracetamol-induced liver damage in rats: The chloroform and methanolic extracts of I. frutescens (CEIF and MEIF) were studied for their hepatoprotective and antioxidant effects on paracetamol (750mg/kg) induced acute liver damage on Wistar albino rats. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein. Further, the effects of both extracts on lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated .CEIF and MEIF at a dose level of 250mg/kg and 500mg/kg produce significant ($P<0.05$) hepatoprotection by decreasing the leves of serum

enzymes, bilirubin, and lipid peroxidation , while they significantly increased the levels of Glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in a dose dependent manner. The effects of CEIF and MEIF were comparable to that of standard drug, Silymarin. From this study, it can be concluded that the chloroform and methanol extract of *I.frutescens* is not only an effective hepatoprotective agent, but also possesses significant ($p<0.05$) antioxidant activity. [63]

55) Prabakaran, et al., (2011) has evaluvated a comparative antimicrobial effect and phytochemical screening of *Plumbago zeylanica, Ocimum gratissimum,Bryophyllum fedtschenkoi*. were subjected to antimicrobial and phytochemical screening. The extraction was taken from these plants using the solvents ethanol, benzene and aqueous. The ethanolic extracts of plant leaves showed significant activity against bacterial and fungal pathogen and results were unpored with standard antibiotics such as *Ampecillin, Penicillin Streptomycin Griscofulevnin, ampotericin* and *fluconazole*. The phytochemical component were identified by using thin layer chromatographic and the results further technique and the supported that some Indian medicinal plants possess. Potential antimicrobial compounds which will be used cure most of the diseases. [64]

56) Vishnu A. Kangralkarl. , et al., (2010) has investigated to elucidate hepatoprotective activity of Methanolic Fruit extract of *Feronia elephantum* in paracetamol induced liver damage in Wistar rats. Liver damage was produced by paracetamol (2gm/kg,p.o.) in 1% CMC. The Plant extract (200mg/kg, p.o.) was administered every 24 hrs for seven days, while standard group received N-acetyl l-cystine . At the end of the study the marker enzymes in serum were analysed. The methanolic extract showed significant hepatoprotective activity and efficacy of extract was almost comparable to that of N-acetyl l-cystine . [65]

57) Vetrivel Rajan , et al ., (2009) has studied the hepatoprotective effects of *Cassia tora* against carbon tetra chloride induced liver damage in albino rats. The efficacy of the treatment was estimated by the serum level marker

enzymes: serum glutamatie oxaloacetic transaminase, serum glumate pyruvate transaminase and lactate dehydrogenase. The treatment so includes the estimation of enzymatic antioxidants superoxide dismutase, glutathione peroxidase, glutathione-S transferase and catalase, non-enzymatic antioxidants: vitamin C and vitamin E. The results of this study increase of marker enzymes in induced rats and decreased level in cassia tora treated ones. Furthermore, the level of enzymatic and non-enzymatic antioxidant level was elevated in treated rats compared to induced ones. [66]

58) Al-Qarawi, et al., (2001) has evaluated the aqueous extract of the *Adansonia digitata* (Linn.) pulp was tested for hepatoprotective activity against chemical toxicity with CCL_4 in rats. The aqueous extract exhibited significant hepatoprotectiveactivity . [67]

59) Manokaran, et al., (2008) conducted to evaluate the hepatoprotective activity of hydroalcoholic extract of *Aerva lanata* against paracetamol induced liver damage in rats. The hydro alcoholic extract of *Aerva lanata* (600mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin . It was concluded from the result that the hydro alcoholic extract of *Aerva lanata* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats. [68]

60) Iniaghe, et al., (2008) of simultaneous treatment of CCl_4 (i.p) with 60 mg/kg (p.o) of aqueous extract of leaves of *Acalypha racemosa* on rat liver was evaluated. Analysis of serum ALT and AST activities with those of the concentrations of albumin, total protein, un conjugated and total bilirubin were carried out. The malondialdehyde (MDA) content of liver was determined to investigate a probable mechanism of action of the extract. Administration of

CCl_4 alone to rats significantly increased total bilirubin concentration and the activities of ALT and AST ($p < 0.05$) in the serum while it significantly reduced ($p < 0.05$) serum total protein and albumin concentrations when compared with controls which received distilled water (p.o). Also it significantly increased ($p < 0.05$) liver MDA content when compared with control. However, simultaneous treatment of CCl4 with 60 mg/kg of the aqueous extract significantly reversed ($p < 0.05$) these changes. Results of MDA content of liver homogenates suggest that a probable mechanism of action of the extract is anti oxidation. Histopathological studies were carried out on the liver to confirm the observed change. [69]

CHAPTER - III

Research envisaged

AIMS AND OBJECTIVES

The objectives of the present investigation are:

1) To prepare hydroalcoholic extract of *Plumbago zeylanica* L. (HAEPZ) and to carry out preliminary phytochemical screening of the extract.
2) To study the anti-inflammatory activity of HAEPZ.
3) To study the analgesic activity of HAEPZ.
4) To study the anti-arthritic activity of HAEPZ.

CHAPTER – IV

Plant profile

PLAN OF WORK

In order to achieve the objectives, following animal models were selected.

1. Anti-inflammatory Activity:

   The anti-inflammatory activity was evaluated using following animal models:

   - ❖ Carrageenan induced rat paw edema.
   - ❖ Cotton pellet granuloma.

2. Analgesic Activity:

   The analgesic activity was evaluated using following animal models:

   - ❖ Formalin induced hyperalgesia.
   - ❖ Hot Plate.
   - ❖ Tail flick.
   - ❖ Acetic acid induced writhing.

3. Anti-arthritic Activity:

   The anti-arthritic activity was evaluated by FCA (Freund's Complete Adjuvant) induced arthritis in rats.

CHAPTER –V

Materials & Methods

PLANT PROFILE

Fig.No:1

Plumbago zeylanica Linn.

SCIENTIFIC CLASSIFICATION

Kingdom	:	Plantae
Unranked	:	Angiosperms
Unranked	:	Eudicots
Unranked	:	Core eudicots
Order	:	Caryophyllates
Family	:	Plumbaginaceae
Genus	:	Plumbago
Species	:	*Plumbago zeylanica*
Synonyms	:	Plumbagoscandens

REGIONAL NAMES

English	:	Lead wort, Ceylon lead wort
Hindi	:	Chira,Chitra.
Gujrati	:	Chitrakmula.
Malayalam	:	Vellakeduveli.
Kannada	:	Chitrakmula,Bilichitrama/ula
Punjabi	:	Chitra
Bengali	:	Chita
Tamil	:	Kodiveli, Chitramoolam
Telugu	:	Chitramulam
Nepali	:	Chitu
Samskrit	:	Chitraka
Arabia	:	Shitaraj, Ensain, Enkin
Assamese	:	Agiyichit, Agmachit ,Bogaagechita
Bengali	:	Chita

Burma	:	Kanchopphiju
Chinese	:	PaiHuaT'eng
French	:	Dentalaire
German	:	Bleiwurz
Indonesia	:	Ceraka
Kashmiri	:	Chitra
Manipuri	:	Chitraka
Oriya	:	Chitamula
Phillipine	:	Sagdikit
Swahili	:	Sanza
Tswana	:	Mosikomabe
Urdu	:	Cheetab

PHYTOCHEMISTRY

Flower: Flowers contain plumbagin, zeylanone, and glucose.

Leaves: Leaves contain plumbagin, chitanone.

Stem: Stem contain plumbagin, zeylanone, isozeylanone, sitosterol, stigmasterol, campesterol, and dihydroflavinol-plumbaginol.

Roots: The root bark of *P. zeylanica* contains plumbagin. The root yield new pigment, viz, 3-chloroplumbagin, 3, 3- biplumbagin, binaphthoquinone identify as 3', 6'- biplumbagin, and four other pigments identify as isozeylanone, zeylanone, elliptinone, and droserone 2, 3. Two plumbagic acid glucosides; 3'o-beta-glucopyranosyl plumbagic acid and 3'-o-beta- glucopyranosylplumbagic acid methyl ester along with five naphthaquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), and five coumarins (seselin, methoxyseselin, suberosine, xanthyletin and xanthoxyletin) were isolated from the roots.

Fruit: It contains plumbagin, glucopyranoside, and sitosterol.

Seeds: Seeds contain plumbagin.

Dr. D. Stephen,
Lecturer
Department of Botany

The American College,
Madurai-2

CERTIFICATE

This is to certify that the plant specimen brought to me by Mr. C. YOGESH PRABHU, IInd year M. Pharm (Pharmacology) Student of **K. M. College of Pharmacy, Madurai** has been identified as ***Plumbago zeylanica Linn*** belonging to the family **Plumbaginaceae.**

Dr.D.Stephen.

Date ; 20/08/2014

Madurai

Tamil nadu

THE AMERICAN COLLEGE MADURAI DEPARTMENT OF BOTANY

CHAPTER –V

Materials & Methods

MATERIALS AND METHODS

Materials

Animals

Species: Swiss albino mice and Wistar rats of either sex were obtained from the K. M. College of Pharmacy, Madurai an approved breeder of laboratory animals.

- Age: Adult – mice and rats.
- Weight: Swiss albino mice- 20-30g, Wistar rats - 180-200g

❖ **Housing conditions:**

Animals were maintained at a temperature of 25±1°C and relative humidity of 45 to 55% under 12-h light:12-h dark cycle. The animals had free access to food pellets and water was available *ad libitum*. They were housed individually in the polypropylene cages after wounding.

❖ **Research protocol approval:**

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India (research protocol approval number no: IAEC/KMCP/129/2014-2015).

Drugs and chemicals

Chemicals:

- ❖ Carrageenan (Sigma-Aldrich, St.Louis MO, USA)
- ❖ Freunds complete adjuvant (Sigma-Aldrich, St.Louis MO, USA)
- ❖ Acetic acid (Pure Chem. Ltd, Pune, India)
- ❖ Pentazocine (Fortwin) Ranbaxy Pharmaceuticals.

- Anaesthetic ether (Batch No. 300) was purchased from TKM Pharma, Hyderabad. Ethanol (GB67890) Batch No. 080618 manufactured by Changshu Chemical.Formalin (Merck)
- Diclofenac (gift sample from Symed labs, Hyderabad).
- Indomethacin (gift sample from Symed labs, Hyderabad).
- 1,1',3,3'-Tetraethoxypropane, Crystalline beef liver catalase, reduced glutathione, 5,5'-dithiobis (2-nitrobenzoic acid), bovine serum albumin, were gift samples obtained from Department of Pharmaceutical Technology, M.S. University of Baroda, India.
- Thiobarbituric acid, tris buffer, sucrose, urethane (Hi-Media Laboratories Pvt. Ltd., Mumbai, India).
- Trichloroacetic acid, citric acid monohydrate, sodium nitrate, copper sulphate, sodium potassium tartarate, methanol, ethanol, ethylene diamine tetra acetic acid disodium salt and Folin's phenol reagent (S.D. Fine Chemicals, Mumbai, India).
- Sodium hydroxide, sodium carbonate, magnesium chloride, sodium carbonate, sodium bicarbonate, Potassium chloride, calcium chloride, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, carbon tetrachloride, chloroform, ether, hydrochloric acid and conc. Sulphuric acid (Qualigens Chemicals Ltd., Mumbai, India)

PHYTOCHEMICAL AND QUALITATIVE ANALYSIS

Extraction of the Plant Material

Collection of plant material

Fresh plant of *plumbago zeylanica Linn* was collected from Alagari Hills, Madurai, Tamil Nadu. The plant specimen was authenticated by Dr.D.Stephen, Lecturer, Dept of Botany, The American College, Madurai, Tamil Nadu.

Preperation of Plant Extract

Materials

- Soxhlet apparatus,
- Pet. Ether, Chloroform, Ethanol, Distilled water and
- Shade dried plant of *Plumbago zeylanica*.

Method

The whole plant of *Plumbago zeylanica* was dried in the shade. Then the shade dried plants were powdered to get coarse powder and about 500gms of dried powder of *Plumbago zeylanica* were soaked in extractor and macerated for 30 hr with petroleum ether, followed by chloroform. Then it is extracted with water: ethanol by continuous hot percolation technique using Soxhlet apparatus for 72hrs. Crude extract were distilled under vaccum condition. After concentration, the hydro alcohol extract gives brownish residue which weigh about 7.2gms. The extract was used for experimentation.

Table No. 1

PHYTOCHEMICAL INVESTIGATION OF EXTRACT OF *PLUMBAGO ZEYLANICA* L.

NAME OF THE TEST	RESULT
TEST FOR CARDIAC GLYCOSIDES	
Legals test	+ ve
Kedde's test	+ ve
TEST FOR ALKALOIDS	
Dragendoff's test	+ ve
Mayers test	+ ve
Wagners test	+ ve
TEST FOR TANNINS	
Ferric chloride test	+ ve

TEST FOR STEROIDS	
Lieberman-Burchard's test	+ ve
Salkowski's test	-ve
TEST FOR FLAVONOIDS	
Ferric chloride test	+ ve
Lead acetate test	+ ve
Sodium hydroxide test	+ ve
TEST FOR SAPONINS	
Frothing test	+ ve
TEST FOR ANTHRAQUINONES	
Brontragers test	+ve
TEST FOR PHOBATINNINS	+ve
TEST FOR CARBOHYDRATES	+ve

Statistical Analysis

The Arithmetic mean ± SEM values were calculated for each experiment. The statistical analyses were carried out with help of Graph pad Prism 5.

CHAPTER –VI

Pharmacological evaluation

PHARMACOLOGICAL EVALUATION

Anti inflammatory activity

❖ **Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Carrageenan induced rat paw oedema (70) Winter *et al*., 1962)**

The male Wistar Rat of 150-200gm Carrageenan induced rat paw oedema is a golden model for screening of acute of anti-inflammatory activity of test compound.

The animals were divided into 5 groups with 6 rats per group as follows-

Group I – Healthy Control

Group II - Vehicle (0.1 % CMC, 10 ml/kg)

Group III - Diclofenac (10 mg/kg)

Group IV - Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (200 mg/kg p.o.)

Group V- Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (400 mg/kg p.o.)

0.1 ml of 1% carrageenan solution was injected into the left hind paw of the rat. The pre treatment time was 1 hour before carrageenan injection. The paw volume was recorded at 0, 1, 2, 3, 4 and 24 hrs by using plethysmometer (UGO Basile 7140). Percentage inhibition of edema was calculated at different hours. The percentage inhibition of edema in the various treated groups was then calculated by using the formula,

$$\% \text{ Inhibition} = (1 - Vt / Vc) \times 100$$

Where, Vt is change in paw volume in the drug treated group

Vc is change in paw volume in the control group.

❖ **Effect of hydroalcoholic extract of *Plumbago zeylanica* L. against cotton pellet granuloma *(71) Swingle and Shideman, 1972)***

Subcutaneous implantation of pellets of compressed cotton provokes foreign body granuloma. The rats were divided randomly into 4 different groups:

Group I - Vehicle (0.5 % CMC, 10 ml/kg)
Group II - Diclofenac (10 mg/kg)
Group III - Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (200 mg/kg p.o.)
Group IV- Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (400 mg/kg p.o.)

Cotton rolls were cut and made into pellets weighing 20 mg each and sterilized in an autoclave at 121°C for 30 min. Four individual pellets were inserted in each ether anesthetized animal by making small subcutaneous incisions in both axillae and groin regions. The incisions were sutured by sterile catgut. After recovery from anesthesia; the animals were treated orally for 7 days. On eighth day animals were sacrificed, and the granulomas were freed from extraneous tissue, dried overnight at 55°c and weighed to obtain constant weight. The average weight of the pellets of the control group as well as of the test group was calculated. The percent change of granuloma weight relative to vehicle control group was determined.

The percentage inhibition of increase in weight of the cotton pellet was calculated as follows:

%Inhibition = (1-(Wt/Wc)) x 100

Wt = Difference in pellet weight of drug treated group
Wc = Difference in pellet weight of control group

Analgesic Activity:

- **Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on hot plate test in mice:**

Male Swiss albino mice (20-25 gm) were divided into four groups containing 6 animals in each group.

Group I - Vehicle (0.1 % CMC, 10 ml/kg)

Group II - Pentazocine (20 mg/kg i.p.)

Group III - Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (200 mg/kg p.o.)

Group IV -Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (400 mg/kg p.o.)

The hot plate test was used to measure the latencies according to the method of Eddy and Leimbach, (1953). Animals were placed on the hot plate maintained at 55 ±1℃, the time between placement of animal on the hot plate and the occurrence of either licking of the fore or hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with basal latencies of more than 20s were eliminated from the study. The testing of response latencies were noted at 0, 30, 60, 90, 120, 150, 180 and 210 min after treatment. The cut off time for hot plate latencies was set at 15s.

- **Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on formalin induced hyperalgesia in mice (72) Murray *et al*., 1988) :**

Male Swiss albino mice (20-25 gm) were divided into four groups containing 6 animals in each group.

Group I - Vehicle (0.1 % CMC, 10 ml/kg)

Group II - Pentazocine (20 mg/kg i.p.)

Group III - Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (200 mg/kg p.o.)

Group IV - Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (400 mg/kg p.o.)

The formalin test was carried out according to the method of **(73) Sugimoto *et al*. (1986) and (74) Ilja Arts *et al*. (2005).** 60 min after the oral or 30 min after intra-peritoneal administration of drugs, 25μL of 5% formalin in saline was subcutaneously injected to the right hind paw of mice, and the nociceptive behavior was recorded for a period of 60 min. The total time (in = seconds) of the nociceptive behavior (licking, biting, lifting and shaking) observed after the injection of formalin was measured as an indicator of nociceptive behavior. The first period (early phase) was 0–10 min after the formalin injection, and the second period (late phase) was 15–30 min after the injection. These phases represented neurogenic and inflammatory pain responses, respectively **(75) Hu WJ et al, 1987).**

- **Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on nociceptive tail flick reflex:**

The tail flick test **(76) D'Amour and Smith, 1941)** is based on tail withdrawal in response to heat from a light beam focused on the ventral tail surface. Tail Flick apparatus (UGO BASILE, Biological Research Apparatus 21025 Camerio VA, Italy) was used. Response latency was the time from application of the light until the tail flicked. The cut off time was 15 seconds. Wistar rats (180-200 g) of either sex were divided into four groups:

Group I - Vehicle (0.1 % CMC, 10 ml/kg)
Group II - Pentazocine (20 mg/kg i.p.)
Group III - Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (200 mg/kg p.o.)
Group IV- Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (400 mg/kg p.o.).

Respective treatment was given to the groups and tail flick latencies of animals was determined at 0, 30, 60, 90, 180 and 210 min after the drug administration.

Anti-arthritic Activity

❖ **Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on FCA induced Arthritis**

Wistar rats (female) weighing 175 to 200 g were divided into five groups of 6 rats each.

Group I – Healthy Control
Group II - Vehicle (0.1 % CMC, 10 ml/kg)
Group III - Indomethacin (10 mg/kg)
Group IV- Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (200 mg/kg p.o.)
Group V- Hydroalcoholic extract of *Plumbago zeylanica* (HAEPZ) (400 mg/kg p.o.).

Two protocols, termed "prophylactic" and "therapeutic" adjuvant arthritis have gained wide usage for assessing a drug potential for antiarthritic activity. In present investigation we have selected the therapeutic schedule for evaluating the anti arthritic effect of the test compound. Wistar rats with an initial body weight of 175-200 gm were used. On day 0, FCA (complete fraction of Mycobacterium butyricum suspended in oil; Sigma Chemical Co., USA) was injected (0.1 ml) into the sub plantar region of the left hind paw. One hundred percent of the animals developed arthritis. Everyday animals were inspected, by examining the affected paw, body weight and the animal's general status. In all the animals, sub plantar injection of FCA produced a pronounced local edema after a few hours with a progressive increase reaching its maximum on the 12^{th} day after inoculation. Dosing with the test compounds was started from day 12 to day 28.

Following parameters were determined:

1. In-vivo parameters

In-vivo parameters were determined on day 0, 3, 7, 10, 12, 14, 17, 21, 24 and 28. The parameters are paw volume, WBC Count, Joint Diameter, Arthritis Score, Body weight, Pain threshold (Randall Sellito), Mechanical Withdrawal Threshold (Von Frey Hair), Paw withdrawal Latency.

2. Biochemical Parameters

On day 28 blood was withdrawn by reto-orbital puncture and serum was used for estimation of Albumin Acid phosphatase and Alkaline Phosphatase.

3. Tissue Estimation:

On day 28 liver tissue was used for the estimation of SOD, MDA, GSH and total protein.

4. Histopatholgy of tibio tarsal joint was performed on Day 28.

- Paw Volume was determined
- WBC count was measured using Neubauer counting chamber.
- Joint diameter was measured by the use of digital vernier calipers.
- Arthritis Score:

The morphological feature of the arthritis like redness, swelling and erythma was monitored daily by set visual criteria. The following scoring system was used:

Normal paw = 0
Mild swelling and erythema of digits = 1
Swelling and erythema of the digits = 2
Severe swelling and erythema = 3
Gross deformity and inability to use the limb = 4

- ❖ Pain Threshold (g) was measured using Randall sellito.
- ❖ Paw withdrawal latency was measured using IR beam using tail flick apparatus.

CHAPTER – VII

Results

RESULTS

Anti-inflammatory Study:

Carrageenan induced rat paw edema:

The paw volume was elevated in carrageenan control when compared to normal control ($p<0.001$). Treatment with hydroalcoholic extract of *Plumbago zeylanica* at a dose of 200 mg/kg and 400 mg/kg exhibited a significant decrease in paw volume at 3rd, 4th and 24th h. HAEPZ (400mg/kg) showed significant ($p<0.001$) decrease in paw volume at 3rd h whereas HAEPZ (200mg/kg) did not show any significant decrease in paw volume at 3h. *Plumbago zeylanica* (200mg/kg) showed significant ($p<0.01$) decrease in paw volume at 4th h. Diclofenac (10 mg/kg) exhibited a significant reduction in paw volume at 2nd h ($p<0.01$) and 3rd, 4th and 24th h ($p<0.001$).

Table No. 2: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on change in paw volume in carrageenan induced paw edema in female wistar rats

Treatment	Change in paw volume (ml)				
	1 h	2 h	3 h	4 h	24 h
Healthy Control	0.07 ±0.00	0.06 ±0.01	0.07 ±0.01	0.06 ±0.01	0.07 ±0.00
Carrageenan Control	0.55 ±0.04#	0.82 ±0.03#	1.61 ±0.09#	1.96 ±0.04#	0.55 ±0.04#
Diclofenac (10 mg/kg)	0.49 ±0.04	0.65 ±0.02**	0.67 ±0.04***	0.45 ±0.03***	0.49 ±0.04***
HAEPZ (200 mg/kg)	0.47 ±0.01	0.74 ±0.04	1.47 ±0.05	1.60 ±0.04**	0.47 ±0.01**
HAEPZ (400 mg/kg)	0.48 ±0.01	0.66 ±0.02	0.72 ±0.06***	0.67 ±0.06***	0.48 ±0.01***

Values are expressed as mean ± SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
**p<0.01, ***p<0.001 when compared to Carrageenan Control
#p<0.001 when compared to Healthy Control

The percentage inhibition of change in paw volume of HAEPZ (200mg/kg) and 400mg/kg was found to be 23.16% and 41.33% respectively at 3h .However the maximum percentage inhibition was found to be at 4h. The percentage inhibition of Diclofenac (10 mg/kg) was found to be 26.86%, 49.23% and 47.73% at 2nd, 3rd & 24thh respectively.

Cotton pellet granuloma

The dry weight of cotton pellet in Diclofenac (4 mg/kg) group after 7 days of treatment was 167.3±7.10 mg as compared to control group (167.3±7.10 mg) ($p<0.001$). Treatment with HAEPZ (200 mg/kg) & HAEPZ (400 mg/kg) resulted in reduced granuloma formation as compared to control group ($p<0.01$ and $p<0.001$ respectively). The percentage inhibition of granuloma formation in HAEPZ 200 and HAEPZ 400 mg/kg was found to be 27.76% and 35.65% respectively.

Table No. 3: Effect of hydroalcoholic extract of *Plumbago zeylanica*. L. on dry weight (mg) in Cotton pellet granuloma in female wistar rats.

Treatment	Mean dry weight of cotton pellet (mg)[1]	Percentage Inhibition (%)
Control	163.3±7.10	--
Diclofenac (4 mg/kg)	78.14±3.40***	41.75
HAEPZ(200 mg/kg)	105.4±3.66**	27.76
HAEPZ(400 mg/kg)	90.58±3.67***	35.65

[1]Values are expressed as mean±SEM of 6 values
[1]One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Control

Analgesic Study

Acetic Acid induced writhing:

The number of writhings in the animals treated with HAEPZ (200 mg/kg) and HAEPZ (400mg/kg) was significantly lower when compared to acetic acid control group ($p<0.05$,$p<0.01$,$p<0.001$ respectively). Diclofenac (10 mg/kg) significantly ($p<0.001$) reduced the no. of writhings when compared to acetic acid control group.

Table No. 4: Effect of hydroalcoholic extract of *Plumbago zeylanica*. L. on no. of writhings in Acetic acid induced writhing in mice

Treatment	No. of writhings
Control	49.67±2.21
Diclofenac (10 mg/kg)	4.833±1.13***
HAEPZ (200 mg/kg)	38.17±2.38**
HAEPZ (400 mg/kg)	25.33±2.52***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Acetic acid Control

Formalin induced hyperalgesia:

Pentazocine reduced the duration of paw licking significantly in phase 1 and phase2 when compared to control group (p<0.001). HAEPZ did not show significant effect in phase1 of formalin induced hyperalgesia. However HAEPZ 200mg/kg and 400mg/kg showed dose dependent reduction (p<0.05, p<0.01, p<0.001 respectively) in duration of paw licking in phase 2 of formalin induced hyperalgesia.

Table No. 5: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on duration of paw licking (sec) in Formalin induced hyperalgesia (Phase I) in mice.

Treatment	Duration of paw licking (sec)
Control	281.7±9.45
Pentazocine (20 mg/kg)	63.83±5.51***
HAEPZ(200 mg/kg)	291.0±8.49
HAEPZ(400 mg/kg)	272.5±10.47

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
***p<0.001 when compared to Normal Control

Table No. 6: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on duration of paw licking (sec) in Formalin induced hyperalgesia (Phase II) in mice

Treatment	Duration of paw licking (sec)
Control	173.2±6.19
Diclofenac (10 mg/kg)	33.33±3.96***
HAEPZ(200 mg/kg)	130.7±5.72**
HAEPZ(400 mg/kg)	62.17±4.72***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
p<0.01, ***p<0.001 when compared to Control.**

Hot plate :

Table No. 7: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Latency (sec) latency in Eddy's Hot plate in mice

Treatment	Latency (sec)						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	3.41 ±0.41	3.23 ±0.30	3.51 ±0.17	3.31 ±0.23	3.76 ±0.34	3.50 ±0.30	3.33 ±0.21
Pentazocine (20 mg/kg)	3.48 ±0.31	5.61 ±0.29***	8.88 ±0.30***	3.91 ±0.21	4.63 ±0.47	3.55 ±0.30	3.23 ±0.21
HAEPZ(200 mg/kg)	3.43 ±0.41	3.55 ±0.17	3.133 ±0.14	4.28 ±0.32	3.60 ±0.24	3.00 ±0.13	3.38 ±0.16
HAEPZ(400 mg/kg)	3.43 ±0.41	3.61 ±0.13	3.40 ±0.23	4.36 ±0.32	3.60 ±0.24	3.05 ±0.17	3.38 ±0.16

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
***p<0.001 when compared to Normal Control

Tail Flick Assay:

HAEPZ did not inhibit thermal hyperalgesia significantly at any of the doses when compared to control group. Pentazocine (20mg/kg ip) significantly inhibited thermal hyperalgesia at 30 & 60 min ($p<0.001$).

Table No. 8: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Latency of tail flick in Tail Flick in female wistar rats.

Treatment	Latency of tail flick (sec)							
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**
Control	4.17 ±0.40	4.17 ±0.30	4.33 ±0.33	4.33 ±0.42	4.35 ±0.50	4.16 ±0.30	3.16 ±0.40	4.00 ±0.36
Pentazocine (20 mg/kg)	4.16 ±0.40	4.16 ±0.30***	4.33 ±0.33***	4.33 ±0.42	3.50 ±0.50	4.16 ±0.30	3.16 ±0.40	4.00 ±0.36
HAEPZ(200 mg/kg)	4.16 ±0.60	6.16 ±0.65	6.50 ±0.42	5.50 ±0.42	4.66 ±0.21	4.66 ±0.33	4.16 ±0.30	4.33 ±0.49
HAEPZ (400 mg/kg)	5.00 ±0.96	4.00 ±0.57	4.00 ±0.57	4.16 ±0.65	5.16 ±0.60	6.16 ±0.47	5.00 ±0.57	4.00 ±0.36

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
***p<0.001 when compared to Normal Control.

Anti-arthritic Study:

Change in paw volume

Change in paw volume of the injected paw of animals of all the treatment group of animals showed a typical biphasic response with an initial peak at 4^{th} day and 12^{th}day post injection of FCA into the sub-plantar region of the rat. Indomethacin (10mg/kg) caused significant ($p<0.001$) inhibition the elevation of the paw volume recorded at 14^{th}, 17^{th}, 21^{st}, 24^{th} and 28^{th} day when compared to arthritic control. On 17^{th} day HAEPZ (200mg/kg) and HAEPZ (400mg/kg) inhibited the rise in paw volume ($p<0.01$,$p<0.001$ respectively). Thus HAEPZ resulted in inhibition of paw volume in dose dependent manner.

Table No. 9: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on change in paw volume (mL) in FCA induced arthritis in female wistar rats.

Treatment	Change in paw volume (mL)									
	0 day	4 day	7 day	10 day	12 day	14 day	17 day	21 day	24 day	28 day
Healthy Control	0.00 ±0.00	0.04 ±0.07	0.04 ±0.08	0.03 ±0.04	0.02 ±0.06	0.04 ±0.12	0.05 ±0.009	0.05 ±0.05	0.05 ±0.02	0.05 ±0.06
Arthritic Control	0.00 ±0.00	2.92 ±0.12#	2.38 ±0.03#	2.92 ±0.12#	3.03 ±0.11#	2.99 ±0.11#	2.92 ±0.11#	2.88 ±0.11#	2.83 ±0.09#	2.50 ±0.06#
Indomethacin (10 mg/kg)	0.00 ±0.00	2.93 ±0.07	2.63 ±0.07	2.90 ±0.03	3.09 ±0.05	2.45 ±0.12***	1.75 ±0.09***	1.19 ±0.05***	0.74 ±0.02***	0.26 ±0.05***
HAEPZ (200 mg/Kg)	0.00 ±0.00	2.92 ±0.03	2.45 ±0.10	2.84 ±0.07	3.04 ±0.01	2.92 ±0.01**	2.57 ±0.05**	2.30 ±0.10**	1.86 ±0.08**	1.64 ±0.10**
HAEPZ (400 mg/kg)	0.00 ±0.00	2.62 ±0.10	2.33 ±0.04	2.60 ±0.09	3.03 ±0.04	2.66 ±0.04	2.18 ±0.04**	1.64 ±0.05**	1.00 ±0.04**	0.87 ±0.11**

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control
#p<0.001 when compared to Healthy Control

Table No. 10: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Percentage Inhibtion paw vloume in FCA induced arthritis in female wistar rats.

Treatment	Percentage Inhibition									
	0 day	**4 day**	**7 day**	**10 day**	**12 day**	**14 day**	**17 day**	**21 day**	**24 day**	**28 day**
Healthy Control	--	--	--	--	--	--	--	--	--	--
Arthritic Control	--	--	--	--	--	--	--	--	--	--
Indomethacin (10 mg/kg)	--	--	--	--	--	34.24	35.83	45.83	47.5	45.75
HAEPZ (200 mg/kg)	--	--	--	--	--	17.16	23.33	33.33	35.17	33.33
HAEPZ (400 mg/kg)	--	--	--	--	--	18.45	36.50	41.50	42.33	41.50

The percentage inhibition of paw volume in animal treated with HAEPZ 400 mg/kg was found to be equal to 30,36.5,41.5,42.33,43.57 respectively on 14^{th} , 17^{th} ,21^{st} , 24^{th} & 28^{th} day. The percentage inhibition in animal treated with HAEPZ 200 mg/kg was found to be equal to 17.17, 23.33, 33.33, 35.17 & 32.86 respectively on 14^{th}, 17^{th}, 21st, 24^{th} & 28^{th} day. Hence HAEPZ was able to inhibit the rise in paw volume in a dose dependant manner during the treatment period starting from 12^{th} day after injection of FCA.

WBC Count

A Change in WBC count of the injected paw of animal of all the treatment group of animals exhibited a typical biphasic response with an initial peak at 4^{th} day and on 14^{th} day. Post injection of FCA into the sub-plantar region of the rat. WBC Count was significantly elevated ($p<0.001$) in arthritic control group when compared to normal control. HAEPZ (200 mg/Kg) and HAEPZ (400 mg/Kg were able to inhibit WBC count in dose dependent ($p<0.01$ and $p<0.001$) manner starting from 17^{th} day.

Table No. 11: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on WBC Count in FCA induced arthritis in female wistar rats.

Treatment	WBC Count									
	0 day	**4 day**	**7 day**	**10 day**	**12 day**	**14 day**	**17 day**	**21 day**	**24 day**	**28 day**
Healthy Control	10298.83 ±132.27	10340.83 ±124.36	10300.67 ±123.88	10302.00 ±117.51	10269.67 ±101.36	10297.33 ±121.68	10241.00 ±77.40	10300.33 ±90.88	10222.30 ±66.83	10251.83 ±68.42
Arthritic Control	10700.00 ±230.94	16003.33 ±409.33#	13166.67 ±362.09#	16003.33 ±409.33#	19616.67 ±162.10#	19516.67 ±160.12#	19150.00 ±164.82#	18600.00 ±175.11#	17983.33 ±199.02#	17700.00 ±177.01#
Indomethacin (10 mg/kg)	10757.50 ±210.03	16087.50 ±611.71	13315.83 ±246.34	16087.50 ±611.70	19506.17 ±219.24	15764.83 ±242.47***	14850.33 ±223.30***	13913.33 ±278.40***	13310.67 ±288.69***	11891.00 ±259.59***
HAEPZ (200 mg/kg)	10838.67 ±226.64	15492.33 ±143.74	13267.33 ±316.55	15492.33 ±143.74	19679.00 ±175.38	18861.17 ±187.97	17891.17 ±427.31**	17221.00 ±392.60**	16573.67 ±365.32**	16414.50 ±329.83**
HAEPZ (400 mg/kg)	10747.17 0±112.21	15380.500 ±180.30	13307.000 ±320.57	15380.500 ±180.30	19658.170 ±179.69	19083.500 ±143.41	17620.670 ±258.73***	16723.170 ±223.24***	15685.170 ±206.75***	15183.670 ±235.28***

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control
#p<0.001 when compared to Healthy Control.

Table No. 12: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on mechanical withdrawal threshold (g) in FCA induced arthritis in female rats

Treatment	Mechanical Withdrawal Threshhold Mean ± SEM									
	0 day	**4 day**	**7 day**	**10 day**	**12 day**	**14 day**	**17 day**	**21 day**	**24 day**	**28 day**
Healthy Control	77.83±2.70	79.66±1.85	80.00±1.96	78.16±2.37	79.00±2.20	79.33±2.55	77.83 ±2.00	78.33 ±2.27	79.83 ±2.37	78.66 ±1.85
Arthritic Control	75.66±1.56	29.00±0.73#	38.83±1.42#	32.16±0.87#	23.33±1.74#	24.00±1.94#	21.16 ±1.83#	21.66 ±1.66#	24.59 ±1.85#	28.00 ±1.63#
Indomethacin (10 mg/Kg)	74.16±2.76	33.33±2.10	39.66±1.99	34.50±1.56	23.16±2.08	41.66±3.01***	49.83 ±2.24***	56.66 ±1.85***	61.00 ±2.22***	70.16 ±1.30***
HAEPZ (200 mg/Kg)	72.33±2.72	34.16±2.52	50.83±2.19	37.33±2.38	23.83±2.21	25.83±2.18	31.50 ±2.06**	36.50 ±2.07**	41.83 ±2.31**	46.00 ±2.11**
HAEPZ (400 mg/Kg)	76.66±3.98	33.66±1.90	47.66±1.45	41.66±1.99	23.50±1.99	27.33±2.02	41.16 ±1.92***	47.83 ±1.30***	51.33 ±1.68***	57.33 ±2.33***

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control
#p<0.001 when compared to Healthy Control

Randall Sellito

Pain threshold was measured by using Randall sellito analgesiometer. Pain threshold reduced in all the treatment groups upto 12^{th} day however after the initiation of treatment pain threshold was elevated. Indomethacin (10mg/kg) was found to significantly elevate ($p<0.001$) the pain threshold in post-treatment period (12^{th}-28^{th} day) starting from 14^{th} day when compared to control group. HAEPZ (200mg/kg) and HAEPZ (400mg/kg) were able to elevate pain threshold when compared to control ($p<0.01$ and $p<0.001$ respectively).

Table No. 13: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on pain threshold (g) in FCA induced arthritis in female wistar rats

Treatment	Pain threshold (g)									
	0 day	**4 day**	**7 day**	**10 day**	**12 day**	**14 day**	**17 day**	**21 day**	**24 day**	**28 day**
Healthy Control	280.00 ±4.47	280.00 ±2.58	276.66 ±4.21	275.00 ±5.62	278.33 ±4.01	268.33 ±7.49	275.00 ±5.53	275.00 ±5.53	270.00 ±6.83	276.66 ±6.14
Arthritic Control	277.500 ±7.71	166.6667 ±4.21#	171.6667 ±4.77#	166.6667 ±4.21#	158.3333 ±6.00#	155.000 ±4.28#	144.1667 ±3.74#	140.000 ±3.65#	131.6667 ±4.01#	145.000 ±5.00#
Indomethacin (10 mg/kg)	270.00 ±5.91	176.66 ±4.94	185.00 ±3.41	180.00 ±3.65	158.33 ±4.77	193.33 ±2.10***	205.00 ±5.62***	228.33 ±4.77***	241.66 ±4.77***	258.33 ±7.49***
HAEPZ (200 mg/kg)	283.33 ±2.47	163.33 ±5.57	176.66 ±3.33	163.33 ±5.57	150.00 ±3.65	185.00 ±4.28	170.00 ±7.74**	181..66 ±3.07**	190.00 ±2.58**	208.33 ±3.07**
HAERPZ (400 mg/kg)	275.00 ±4.28	171.66 ±3.07	178.33 ±3.07	171.66 ±3.65	150.00 ±4.28	185.00 ±6.00	190.00 ±4.42***	211.66 ±6.00***	216.66 ±3.33***	230.00 ±5.16***

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control
#p<0.001 when compared to Healthy Control.

Table No. 14: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Arthritis Score in FCA induced arthritis in female wistar rats.

Treatment	Arthritis Score									
	0 day	**4 day**	**7 day**	**10 day**	**12 day**	**14 day**	**17 day**	**21 day**	**24 day**	**28 day**
Healthy Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Arthritic Control	0.00±0.00	2.50±0.22$^{\#}$	2.66±0.21$^{\#}$	3.83±0.16$^{\#}$	4.00±0.00$^{\#}$	4.00±0.00$^{\#}$	3.83±0.16$^{\#}$	3.83±0.16$^{\#}$	3.83±0.16$^{\#}$	3.83±0.16$^{\#}$
Indomethacin (10 mg/kg)	0.00±0.00	2.83±0.16	2.66±0.21	3.33±0.21	4.00±0.00	1.66±0.21***	1.33±0.33***	0.83±0.30***	0.66±0.21***	0.33±0.21***
HAEPZ (200 mg/kg)	0.00±0.00	2.83±0.16	2.83±0.21	3.33±0.21	4.00±0.00	3.16±0.16	2.50±0.22**	2.16±0.16**	1.66±0.21**	1.50±0.22**
HAEPZ (400 mg/kg)	0.00±0.00	2.66±0.00	2.33±0.21	3.66±0.21	4.00±0.00	2.50±0.22	2.16±0.16***	1.66±0.21***	1.16±0.16***	0.83±0.16***

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control
#p<0.001 when compared to Healthy Control

Table No. 15: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on paw withdrawal latency (sec) in FCA induced arthritis in female wistar rats

Treatment	Paw withdrawal latency (sec)									
	0 day	**4 day**	**7 day**	**10 day**	**12 day**	**14 day**	**17 day**	**21 day**	**24 day**	**28 day**
Healthy Control	11.53±0.33	11.90±0.13	11.95±0.15	12.03±0.08	11.53±0.33	11.90±0.13	11.88±0.13	11.90±0.13	11.95±0.15	12.05±0.07
Arthritic Control	11.53±0.33	4.06±0.29$^{\#}$	6.01±0.24$^{\#}$	4.06±0.29$^{\#}$	2.30±0.21$^{\#}$	2.16±0.15$^{\#}$	2.06±0.12$^{\#}$	1.75±0.21$^{\#}$	1.66±0.17$^{\#}$	2.85±0.25$^{\#}$
Indomethacin (10 mg/Kg)	11.60±0.23	3.78±0.09	5.58±0.26	3.78±0.09	2.53±0.17	4.93±0.41***	6.83±0.40***	8.80±0.23***	9.65±0.18***	10.65±0.19***
HAEPZ (200 mg/Kg)	11.76±0.08	3.316±0.16	5.60±0.31	3.31±0.16	2.25±0.17	2.58±0.18	3.51±0.23**	4.21±0.25**	5.10±0.23**	6.20±0.25**
HAEPZ (400 mg/Kg)	11.90±0.04	3.38±0.19	5.80±0.25	3.38±0.19	2.26±0.15	2.83±0.23	4.38±0.23***	5.55±0.34***	6.51±0.38***	8.41±0.35***

Values are expressed as mean±SEM of 6 values
Two way ANOVA followed by Bonferroni post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control
#p<0.001 when compared to Healthy Control

Paw Weight

Table No. 16: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Paw weight (g) in FCA induced arthritis in female wistar rats.

Treatment	Paw weight (g)
Healthy Control	141.5±4.16
Arthritic Control	228.0±4.30
Indomethacin (10 mg/kg)	146.8±5.00***
HAEPZ (200 mg/kg)	186.5±4.07***
HAEPZ (400 mg/kg)	166.7±4.41***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
***p<0.001 when compared to Arthritic Control.

Paw weight was elevated in arthritic control group. Indomethacin (10 mg/kg) significantly reduced the rise in paw weight when compared to arthritic control animal (p<0.001). HAEPZ (50 mg/kg) did not show any significant effect on rise in paw weight when compared to control. HAEPZ (200 mg/kg) & HAEPZ (400 mg/kg) exhibited significant reduction in paw weight when compared to arthritic control (p<0.01&p<0.001respectively).

Serum Albumin

Table No. 17: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Serum Albumin (g/dl) in FCA induced arthritis in female wistar rats.

Treatment	Serum Albumin (g/dl)
Healthy Control	4.36±0.11
Arthritic Control	2.18±0.08***
Indomethacin (10 mg/kg)	4.01±0.09**
HAEPZ (200 mg/kg)	2.83±0.19**
HAEPZ (400 mg/kg)	3.63±0.09**

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control.

Serum albumin levels were reduced significantly in arthritic control group. Indomethacin (10 mg/kg) significantly elevated (p<0.001) serum albumin levels when

compared to arthritic control group HAEPZ 200 & HAEPZ 400 mg/kg exhibited dose dependent elevation of serum albumin level when compared to arthritic control ($p<0.05$, $p<0.01$, $p<0.001$).

Serum Alkaline Phosphatase

Serum alkaline phosphatase was elevated in the arthritic control group. The rise in serum alkaline phosphatase was significantly inhibited ($p<0.001$) in the animal treated with Indomethacin (10 mg/kg). The rise of Serum alkaline phosphatase was inhibited in the dose dependent manner in animal treated with HAEPZ 200 & HAEPZ 400 mg/kg ($p<0.05$, $p<0.01$ and $p<0.001$ respectively).

Table No. 18: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Serum Alkaline Phosphatase (U/L) in FCA induced arthritis in female wistar rats.

Treatment	Serum Alkaline Phosphatase (U/l)
Healthy Control	249.5±10.13
Arthritic Control	915.7±42.80***
Indomethacin (10 mg/kg)	283.8±16.64***
HAEPZ (200 mg/kg)	787.2±17.04**
HAEPZ (400 mg/kg)	411.0±14.26***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control

Serum Acid Phosphatase

Table No. 19: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Serum Acid Phosphatase (U/l) in FCA induced arthritis in female wistar rats

Treatment	Serum Acid Phosphatase (U/l)
Healthy Control	3.81±0.17
Arthritic Control	8.91±0.23***
Indomethacin (10 mg/kg)	3.90±0.23**
HAEPZ (200 mg/kg)	5.71±0.38***
HAEPZ (400 mg/kg)	4.61±0.20***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control

Serum acid phosphatase was elevated in the arthritic control group. The rise in serum acid phosphatase significantly inhibited ($p<0.001$) in the animal treated with indomethacin (10 mg/kg) . The rise of serum acid phosphatase was inhibited in the dose dependent manner in animal treated with HAEPZ 200 & HAEPZ 400 mg/kg ($p<0.05$, $p<0.01$ & $p<0.001$ respectively).

Oxidative Stress in Liver

MDA

Table No. 20: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on MDA (nMol/mg of protein) in FCA induced arthritis in female wistar rats.

Treatment	MDA (nmol/mg of protein)
Healthy Control	2.64±0.35
Arthritic Control	7.87±0.43
Indomethacin (10 mg/kg)	3.61±0.23***
HAEPZ (200 mg/kg)	4.9±0.13**
HAEPZ (400 mg/kg)	3.64±0.29***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control

The level of MDA was elevated in the Arthritic Control group (7.87±0.43) as compared to the healthy control group (2.64±0.35). Indomethacin significantly inhibited ($p<0.001$) the rise of MDA. The rise of MDA was inhibited in the dose dependent manner in animal treated with HAEPZ 200 & HAEPZ 400 mg/kg ($p<0.05$, $p<0.01$ & $p<0.001$) respectively.

(ii) Glutathione (GSH)

The level of glutathione in the Arthritic control group was reduced to 16.00±1.01 from the normal value of 29.58±1.24. Indomethacin (10 mg/kg) significantly increased ($p<0.001$) the level of glutathione. The glutathione levels was increased in the dose dependent manner in animal treated with HAEPZ 200 & HAEPZ 400 mg/kg ($p<0.05$, $p<0.01$ and $p<0.001$) respectively.

Table No. 21: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on Blood Glutathione (µg/mg) of protein in FCA induced arthritis in female wistar rats

Treatment	GSH (µg/mg of protein)
Healthy Control	29.58±1.24
Arthritic Control	16.00±1.01
Indomethacin (10 mg/Kg)	27.15±0.74***
HAEPZ **(200 mg/Kg)**	24.11±1.31**
HAEPZ **(400 mg/Kg)**	24.75±0.93***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control

(iii) SOD :

The level of SOD in the vehicle treated group was reduced to 2.65±0.34 U/l from the normal value of 11.23±0.35 U/l. Indomethacin (10 mg/kg) significantly increased (p<0.001) the level of glutathione. The glutathione levels was increased in the dose dependent manner in animal treated with HAEPZ 200 & HAEPZ 400 mg/kg (p<0.05, p<0.01 & p<0.001) respectively

Table No. 22: Effect of hydroalcoholic extract of *Plumbago zeylanica* L. on SOD (U/mg protein) in FCA induced arthritis in female wistar rats.

Treatment	SOD (U/mg of protein)
Healthy Control	11.23±0.35
Arthritic Control	2.65±0.34***
Indomethacin (10 mg/kg)	9.39±0.56**
HAEPZ **(200 mg/kg)**	5.22±0.29***
HAEPZ **(400 mg/kg)**	8.42±0.34***

Values are expressed as mean±SEM of 6 values
One way ANOVA followed by Dunnet's post-test
**p<0.01, ***p<0.001 when compared to Arthritic Control

Histopathology Results

Normal connective tissue of tibio tarsal joint.
No necrosis present.
No lymphocytic infiltration present.

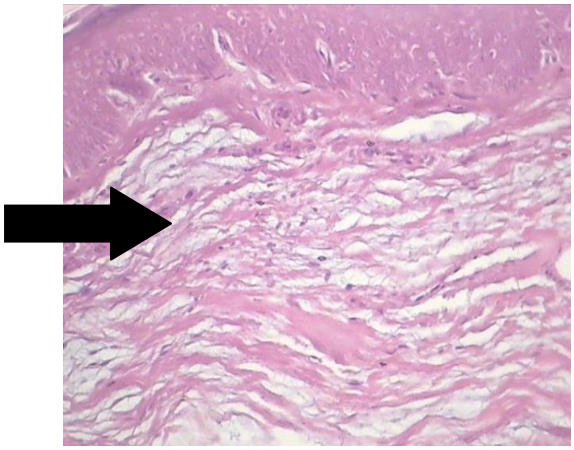

Fig. 2 Histopathological representation of tibio tarsal joint of healthy control animal.
Stain: H & E
Thickness: 5µ
Magnification: 40x

Inflamed connective tissue of tibio tarsal joint.
Necrosis present.
Lymphocytic infiltration present.

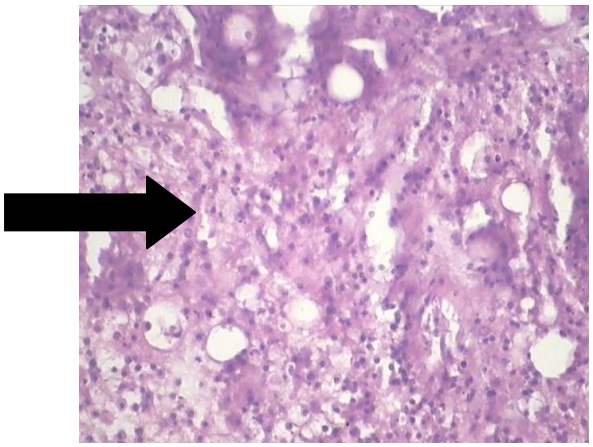

Fig. 3 Histopathological representation of tibio tarsal joint of arthritic control animal.
Stain: H & E
Thickness: 5µ
Magnification: 40x

Normal connective tissue of tibio tarsal joint.
No necrosis present.
No lymphocytic infiltration present.

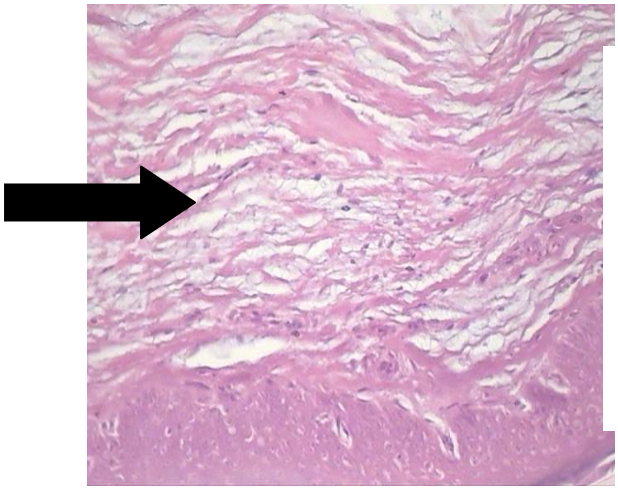

Fig. 4 Histopathological representation of tibio tarsal joint Indomethacin (10 mg/kg) treated animal.
Stain: H & E
Thickness: 5µ
Magnification: 40x

Inflamed connective tissue of tibio tarsal joint. Mild necrosis present. Mild lymphocytic infiltration present.

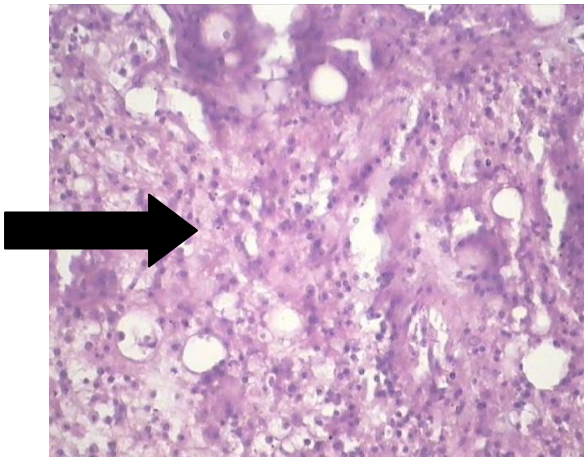

Fig. 5 Histopathological representation of tibio tarsal joint HAEPZ (200 mg/kg) treated animal.
Stain: H & E
Thickness: 5µ
Magnification: 40x

Normal connective tissue of tibio tarsal joint. No necrosis present. No lymphocytic infiltration present.

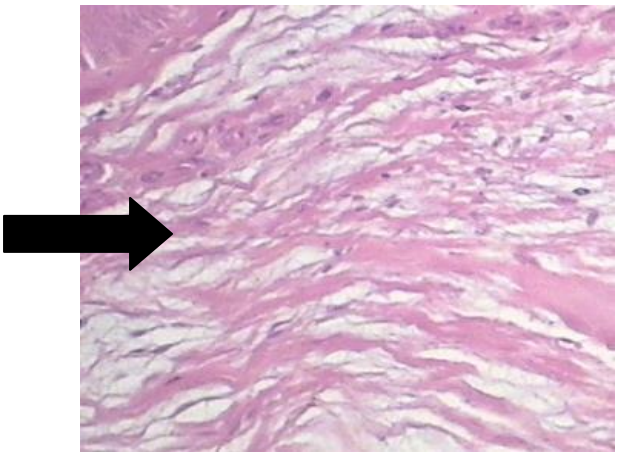

Fig. 6 Histopathological representation of tibio tarsal joint HAEPZ (400 mg/kg) treated animal.
Stain: H & E
Thickness: 5µ
Magnification: 40x

The histopathological evaluation of the tibio tarsal joint show prominent inflamed degenerative connective tissue associated with cellular inflammation edema, granuloma formation in the vehicle treated animal. However these changes were not observed in the animal treated with Indomethacin (10 mg/Kg). Mild histopathological changes were observed in HAEPZ (200 mg/kg) and no histopathological changes were observed in HAEPZ (400 mg/kg) treatment groups respectively. However bone disruption erosion deformation was not observed.

CHAPTER – VIII

Discussion

DISCUSSION

Plumbago zeylanica L. is an Indian garden shrub with potent medicinal properties. The present investigation is undertaken to find its analgesic, anti-inflammatory and anti-arthritic activity of hydroalcoholic extract of *Plumbago zeylanica* L.

The anti-inflammatory activity was performed using carrageenan induced rat paw edema. Carrageenan is a sea weed which serves as a phlogistic substance when injected locally into the sub-plantar region. The acute severe inflammation is morphologically visible which is evaluated by paw volume. The paw edema induced by sub-plantar injection is biphasic. The early phase involves the release of serotonin, histamine and kinins, while the late phase is mediated by prostaglandins **(77) Vinegar *et al*., 1969).** In the present investigation there was an increase in the paw volume upon injection of carrageenan as observed at 1h, 2h, 3h,4h and 24h. Pre-treatment with hydroalcoholic extract of *Plumbago zeylanica* L. 200 and 400 mg/kg decrease the paw volume at 3h and 4h. Histamine is released at 1h. Serotonin and bradykinin are released at 2h and prostaglandins are released at 3h. Thus the observed decrease in paw volume might be due to inhibition of prostaglandins.

Insertion of cotton pellet forms granuloma which is a sub-acute inflammatory condition. This leads to tissue proliferation and granuloma formation. This method is widely employed to assess transudative, exudative and proliferative components. The dry weight of the granuloma correlates with the amount of granulomatous tissue formed. In the present study dry weight granuloma has significantly reduced with HAEPZ 200 and 400 mg/kg treated groups which indicate that HAEPZ at higher doses is effective in alleviating inflammation.

Hot plate and tail flick is used to evaluate centrally acting analgesics. In the present study there was no significant change brought about by HAEPZ in any of these models which indicate HAEPZ is devoid of central analgesic activity.

Formalin is a phlogistic substance which creates localized inflammation and hyperalgesia when injected in the sub-plantar region. It shows a biphasic response due to neurogenic inflammation (neurogenic pain) followed by participation of kinins, leukotriene and prostaglandins. The first phase (0-5 min) is the neurogenic phase during which the peripheral nociceptors are stimulated acts on chemoreceptor in the nociceptor neuron leading to acute phase. The second phase lasts from 20-30 min which represents the inflammatory phase mediated by kinin, leukotrienes and prostaglandins. In the present investigation, HAEPZ inhibited the second phase in a dose dependent manner but was ineffective in amelioration of the pain in the first phase which indicate that HAEPZ does not have inhibitory effect on the release of substance P from dorsal root ganglia which is the primary site of central pain. However the ability of HAEPZ to inhibit the second phase indicate inhibitory effect of HAEPZ on COX pathway.

Acetic acid is a phlogistic substance which causes localized inflammatory response manifested as writhing when injected intra-peritoneally into mice. This is a sensitive method for screening peripherally acting analgesic compounds. Acetic acid causes release of mediators of inflammation like bradykinin, serotonin, PGE_2 and $PGE_{2\alpha}$ in the peritoneal fluid which reduce the pain threshold and induces a state of hyperalgesia. In the present investigation HAEPZ inhibited the acetic acid induced writhing in a dose dependent manner.

The peripheral analgesic activity of acetic acid may be because of inhibition of inflammatory mediators which is evident through acetic acid induced writhing and formalin induced hyperalgesia models. The ability of HAEPZ to inhibit the COX pathway is also established through carrageenan induced paw edema model.

FCA induced arthritis is a well established model which mimics human pathological state. This model was pioneered by Pearson and Wood (1963). FCA comprises of heat killed Mycobacterium tuberculi suspended in paraffin oil. This is a widely used animal model which represents chronic immune mediated joint inflammation that is induced by intradermal or subcutaneous injection of FCA. Ensuing polyarthritis represents a localised inflammatory and systemic disease. Symptoms are visible not only in the injected paw but in ankle, wrist, tarsal, carpal

and inter-phalangeal joint. FCA arthritis follows a biphasic time course consisting of an acute inflammatory condition which peaks at 3 to 5 days and a chronic systemic reaction that shows a relaxing remitting course persisting for several weeks **(78) Schaible *et al*., 2001 , (79) Bendele 2001 , (80) Seino *et al*., 2006).** The immunologically mediated FCA arthritic model of chronic inflammation is considered as the best experimental model of rheumatoid arthritis **(81) Williams 1998).** The bacterial peptide glycan and muramyl dipeptide and important nonspecific immunogenic components **(82) Crofford and Wilder, 1993)** Adjuvant arthritis with or without an autoimmune component induced by compound which does not contain MHC binding peptide but involves T cell activation intra-dermal or subcutaneous injection of Freund's adjuvant produce a chronic relapsing arthritis with characteristics of rheumatoid arthritis . In this model immunologically mediated chronic synovial inflammation and macrophage play a central role. After activation they are capable of synthesising mediator such as PGE_2 and cytokinin such as TNFα and Intelukin–1. In turn their synthetic product induce the production of variety of enzymes which initiate cartilage and bone destruction **(83) Hopkins, 1990, (84) Jadot *et al*., 1986).** In the chronic inflammation, activated immune system can release a series of pro-inflammatory mediators cytokinin, including tumour necrosis factor (TNF α).

Several cytokines (TNF α IL-1, IL-6, and GM-CSF) and activated β cells and T cells play a crucial role in RA joint inflammation. The persistence of inflammation in established RA is driven by interaction between T cells, macrophages and fibroblasts in an abnormal microenvironment. The synovial T cell population is maintained through active inhibition of apoptosis, mediated at least in part by fibroblast and macrophage derived type 1 TNFs and active retention facilitated by fibroblast derived transforming growth factor β. Contact dependent interaction between T cell and macrophages stimulate the production of pro-inflammatory cytokinins including TNFα in an antigen independent manner . Cytokines secreting T cells may indeed play a role in an inflammatory synovitis both by initiating and maintaining the disease process **(85) Raza *et al*., 2005).** Progression of RA is associated with an imbalance of Th1/Th2 and overproduction of antigen specific immunoglobulin **(86) Panayi, 1997).**

These inflammatory mediators produce profound changes in the injected paw which can be evaluated using various parameters like change in paw volume ,change in joint diameter, WBC count, pain threshold (in grams in randall sellito), arthritis score, paw withdrawal tendency, serum albumin, serum acid phosphate, oxidative stress in liver due to arthritis, paw weight, and histopathology. In the present investigation the change in paw volume during the entire treatment period was monitored and percentage inhibition was determined at various time points in all the treatment group of animals. The rise in paw volume was inhibited in dose dependent manner proving the ability of HAEPZ to inhibit the inflammatory, oxidative changes which occur in FCA induced arthritis. Synovitis of the tibio tarsal joint was inhibited in the animals treated with HAEPZ depicting the ability of HAEPZ to inhibit immune mediated joint inflammation and synovial hyperalgesia and accumulation of neuterophil. The joint diameter of the HAEPZ treated animals was not elevated when compared with the vehicle treated control group animals.

FCA induced arthritis leads to peripheral pain and hyperalgesia. The neurons in the injected paw become sensitive to allodynia and hyperalgesia induced by nociceptive agents which are measured using randall sellito. Rats with severe arthritis in the vehicle treated group demonstrated low paw withdrawal threshold. The animals treated with HAEPZ are able to bear significantly higher pressure while subjected to Randall Sellito analgesiometer whereas the animals in the control group were able to bear the pressure demonstrated as a very small latency and minimum weight bearing capacity due to severe arthritic condition. Randall sellito throws light on the weight bearing hyperalgesic response. In the present investigation HAEPZ was able to alleviate both the pain responses in the plantar region of the rats in various treatment groups.

Thermal hyperalgesia is an important parameter used to evaluate the disease condition in FCA induced arthritic rats. In arthritic condition the thermo receptors located at the injected paw are stimulated at a lower threshold than normal animal or the non-injected paw. Hence the paw withdrawal latency in the ipsilateral paw of the animals treated with HAEPZ at all the three doses showed prolong latency period when compared with the vehicle control group of animals .These investigations are in accordance with the study done using acetic acid as the phlogistic substance leading

to nociceptive condition. Hence the anti-inflammatory and peripheral analgesic property of HAEPZ is well explained via a plethora of in-vivo tests.

The WBC count in the synovial fluid and the circulating blood is elevated in inflammatory condition of the total WBCs count was elevated in the control group whereas in the HAEPZ treated group the elevation of WBC count was inhibited. This provides credence to the fact that HAEPZ is able to not only to reduce the inflammatory condition at the systemic circulation.

Paw weight corresponds to the accumulation of exudate in the injected paw leading to elevation of weight. The various inflammatory mediators cause accumulation of lymphocytes, macrophages and other inflammatory mediators at the site of injection leading to increase in the weight of the paw. In the present investigation the elevation of paw weight was found to be inhibited in the HAEPZ treated group in a dose dependent pattern which further supports the anti-inflammatory profile of HAEPZ.

Histopathological studies also show severe exudative changes and infiltration of nuetrophils and macrophages in the inflamed tissue when section 0f 5µ was made and stained with hematoxylene and eosin. However these changes were absent in the HAEPZ 200mg/kg and indomethacin treated animals.

RA is not only restricted to paw but it is also systemic disease which affects the overall oxidative stress in the animal. This fact is an indispensable and inevitable factor which needs to be thoroughly investigated. The oxidative stress is visible in the synovial tissue and liver of the arthritic animal. The serum levels of ACP, Albium, ALP are elevated in arthritic animals. In the present investigation HAEPZ was able to venture the elevated and reduced level of ACP, ALP and albumin in the animals treated with HAEPZ in a dose dependent manner. Serum alkaline phosphatase and acid phosphtase was significantly increased in the control group of animals. However the elevated levels of ACP and ALP were reduced in the animals treated with HAEPZ in a dose dependent manner.

Oxidative stress plugs a pivoted role in modulating the disease state in arthritis FCA induced arthritic model demonstrates a severe rise in the various cytotoxic free radicals in plasma joint as well as liver **(87) cerhan *et al*., 2003 , (88) Rajkapoor *et al*., 2009)**. In adjuvant induced arthritis lipid peroxidation leads to tissue damages and is associated with the aggravation of arthritis **(89) Halliwell *et al*.,1988).** Lipid peroxidation causes lysosomal destruction which also evolves in arthritis development hence the level of these enzymes in the liver serves as the representation of the oxidative stress in the entire body of the animal. Hence SOD, GSH and MDA were measured in the liver of the rats in the various treatment groups. Glutathione is endogenously synthesized in the liver and serves as first line of defense against oxidative stress and peroxidation **(90) Rasool and Varalakshmi, 2009).**

The role of NO has been well established in an anti-inflammatory response. As the inflammatory response progresses, large quantities of NO are generated through the induction of iNOS (inducible Nitric oxide synthase) that reacts with superoxide anion to form peroxynitrate a potent oxidizing molecule capable of eliciting lipid peroxidation. Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids to form radical intermediates that bring about cellular damage. MDA, a major end product of this reaction, is an index of lipid peroxidation and has been estimated as TBARS besides, the infiltrating cells also generate reactive oxygen species and free radicals that brings about destruction of the inflamed joint. As a result, the scavenging enzyme SOD that leads to the formation of hydrogen peroxide is utilized and its activity is reduced in arthritic rats. The hydrogen peroxide thus generated is decomposed by catalase and glutathione peroxidase. Excessive production of lipid hydrogen peroxide may also contribute to decreased activity of GPx in arthritic condition. Beside enzymatic antioxidants, the level of glutathione, a non-enzymatic reducing agent that traps free radicals and prevents oxidative stress, is also decreased in arthritis. The most reactive oxygen species in biological system are superoxide anion, hydroxyl radical and hydrogen peroxide **(91) Badgujar *et al*., 2009).** Superoxide dismutase can change superoxide anion radical to hydrogen peroxide and catalase cleaves this hydrogen peroxide into the molecules of water and oxygen. Glutathione peroxidase is also a detoxifying enzyme, changing the peroxides to water **(92) Lawrence and burk, 1976).** In the CFA reagent induced rheumatoidal model, the increase of lipid peroxide could be attributed not only to the activation of

xanthine oxidase and aldelyde oxidase but also to the deactivation of superoxide dismutase, catalase and selenium-dependent glutathione peroxidase. In the present investigation, the level of oxidative stress markers in the liver was determined at the end of the study. HAEPZ was able to inhibit the oxidative changes brought about by FCA. The elevated MDA levels in the control groups were reduced where as the reduced SOD and GSH levels were elevated to restore normal level of oxidative markers in the livers. This response was dose dependent. It could be postulated that the amelioration of arthritis was due to the suppression of oxidative stress in the liver of the animals. It is to be noted that *Plumbago zeylanica* L. has been proven to ameliorate myocaradial ischemic reperfusion injury by inhibiting lipid peroxide SOD, catalase and glutathione.

CHAPTER – IX

Conclusion

CONCLUSION

It could be concluded from present investigation that HAEPZ possesses potent anti-inflammatory, peripheral analgesic and anti-arthritic property. It was able to inhibit acute inflammatory response in various animal models & was able to ameliorate arthritic changes in the FCA induced RA model in rats. It also exhibited a potent antioxidant profile by inhibition of generation of ROS and decrease in oxidative stress. These observations provide pharmacological credence to ethnobotanical claims of traditional Indian system of medicine. This study proves for the first time anti-arthritic potential of HAEPZ. The study would contribute to develop novel herbal formulations which will pave the way for explorations of novel vistas in the field of anti-inflammatory drugs.

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